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Abstracts

EDITORIAL PREFACE

Most of the specialities of medical science are represented in the extensive Scandinavian medical literature by journals devoted to their particular subjects. These journals, which to a large extent must limit their scientific content to the sphere of interest of the particular speciality they represent, have become firmly established through the years, and each now forms a natural nucleus for the scientific life within its own branch of Scandinavian medicine.

In addition to this type of journal, there is also a need for a journal devoted to experimental clinical research common to all specialities — a journal, the content of which is selected on the basis of the manner in which the particular scientific problem is approached, regardless of which particular field of medical science it belongs to. The "Scandinavian Journal of Clinical and Laboratory Investigation" is designed to fulfill this need. The scientific material which will be published in the journal must be closely associated with clinical research and must be based on laboratory investigations. Research executed on the basis of clinical statistics and casuistics will thus fall outside the scope of the journal. On the other hand, it is self-evident that methodological work of importance for clinical research should find a place in a journal of this character.

The journal will also include a short practical section where technical details, short surveys of subjects important from a practical standpoint, description and evaluation of new apparatus etc., will be published.

It is the hope of the Editorial Board that "The Scandinavian Journal of Clinical and Laboratory Investigation" will be able to accomplish the task outlined above and in this way help to support the evolution and progress of clinical laboratory activity and assist in the promotion of experimental clinical medicine within the Scandinavian countries.

COMPLETE PORPHYRIN ANALYSIS
OF PATHOLOGICAL URINES[1]

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Biochemical study of the porphyrino-pathies is handicapped by the lack of suitable methods for the quantitative determination of uroporphyrin isomers in the excreta. Normal urine contains some 20—100 µg/diem of coproporphyrin (isomers I and III) but the uroporphyrins are absent or present only in traces (Fischer & Zerweck, 1924). In pathological conditions, the quantity of coproporphyrin may be increased without any appearance of uroporphyrin (porphyrin-uria), excretions of 1 mg. and more/diem having been recorded in severe hepatic disease and in lead poisoning. Such increase in porphyrin excretion is to be regarded as purely symptomatic. The group of conditions, however, to which the term porphyria has been applied (Waldenström, 1937) are characterized by the excretion in the urine of quantities of the order of 1—60 mg/diem of uroporphyrins or their precursors, the coproporphyrin excretion being, at the same time, only slightly or moderately elevated.

A familial incidence or predisposition is evident in these cases.

Waldenström (1937) emphasizes the distinction between congenital porphyria, present from birth and probably inherited as a mendelian recessive and acute porphyria (with its subdivisions) and also porphyria cutanea tarda which are dominant in character, appear later in life and usually follow a course of repeated exacerbation and remission.

There are also chemical differences to be noted. Whereas in congenital porphyria the uroporphyrin produced is mainly of series I together with some series III (Rimington 1936, Fischer & Hoffman 1937) the urine of acute porphyria contains a substance porphobilinogen (Waldenström 1937, Waldenström & Vahlquist 1939) which is not encountered in any other of the porphyrinopathies and which, under appropriate conditions, is transformed into a mixture of uroporphyrin isomers I and III in which type III predominates.

Grinstein, Schwartz & Watson (1945) Watson, Schwartz & Hawkinson (1945) and Prunty (1946) have seriously questioned the individuality of Waldenström's

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uroporphyrin III, nevertheless, in the present paper, when considering its quantitative determination, it will be referred to as a single individual.

The coproporphyrins are extractable from aqueous media by ether containing acetic acid (Saillet 1896) and are thus readily determined by Fischer's (1926) wellknown procedure. Uroporphyrin III was shown by Waldenström, Fink & Hoerbürger (1935) to be extractable from solutions at pH 3.0 —3.2 by ethyl acetate but uroporphyrin I is not extractable by any solvent under known conditions, and its determinations is, therefore, a matter of very great difficulty.

Rimington (1943) proposed a method for the determination of total porphyrin, including uroporphyrins I and III, which depended upon adsorption of the porphyrins from acidified urines onto kieselguhr, their subsequent elution by repeated washing with $0.1\ N$ — sodium hydroxide and measurement of the fluorescence intensity in this solvent. Subtraction of the separately determined coproporphyrin gave a figure for the combined uroporphyrin content.

This method suffered from the disadvantages that it was tedious (particularly the elution with NaOH) and on account of the variability of specimens of kieselguhr.

A greatly improved technique has now been worked out, which permits of the separate determination of coproporphyrin (Fischer's method of extraction followed by spectrophotometry), total uroporphyrin (adsorption and spectrophotometry) and uroporphyrin III (ethyl acetate soluble fraction) whence any uroporphyrin I may be obtained by difference. If desired, the coproporphyrins, also, may be divided into isomers I

and III by the procedure of Watson & Schwartz (1940) or of Schwartz, Hawkinson, Cohen & Watson (1947). A complete quantitative porphyrin analysis of pathological urines thus becomes possible.

METHODS

Porphyrins used as standards and in recovery experiments were all derived from natural sources. They were all purified by recrystallization as the methyl esters from chloroform : methanol mixtures and for use in the free state were hydrolysed in either acid or alkaline solution.

Spectrophotometric measurements were all made on the Beckman photoelectric spectrophotometer. For data concerning specific adsorptions of the uroporphyrins see Rimington & Sveinsson (forthcoming publication).

EXPERIMENTAL

Adsorbents for porphyrins.

The porphyrins are readily adsorbed from pure solutions by a number of different adsorbents but adsorption from biological fluids such as urine is often disappointing from the quantitative aspect. Moreover, to be of practical use the adsorbed pigment should be easily and quantitatively recovered by elution but this is often difficult of achievement.

Alumina as manufactured "nach Brockman" (Merck) or "for adsorption purposes" (Savoury & Moore Ltd.) has frequently been used for the quantitative removal of porphyrins from urine. Filtration through columns of the adsorbent is always slow and the rate rapidly diminishes so that only relatively small volumes of urine can conveniently be handled by this method. Elution is usually performed by dilute ammonia but is rarely if ever quantitative.

Kieselguhr will adsorb porphyrins from acid solutions and reject considerable quantities of the dark brown pigments which frequently accompany porphyrins in porphyria urines (Rimington 1943). Nevertheless, it suffers from the disadvantages of being variable in activity and tedious to handle during the elution procedure.

Calcium carbonate and *magnesium hydroxide* are both excellent adsorbents for porphyrins from

pure solution but usually fail when applied to urine. The reason for this became clear after our study of the calcium phosphate precipitates (see below).

Calcium phosphate, as formed in urine when the latter is made alkaline, has been employed by Garrod (1894—95) and others subsequently for the concentration of urinary porphyrins but it was recognized that adsorption was variable in degree and rarely if ever quantitative. Dobriner (1936) in his scheme for the separation of urinary porphyrins recommends the addition of calcium chloride to the urine before alkalinization, presumably to increase the bulk of the precipitate, since no other reason is given. The phosphate precipitate has the advantage that it is easily redissolved in acids and the porphyrin thus set free.

In our own experiments we decided to investigate the relation between composition of the precipitate or precipitating system and affinity for porphyrin. Addition of dry, solid $CaHPO_4$ or $Ca_3(PO_4)_2$ (commercial specimens) to a solution of uroporphyrin III in very dilute ammonium hydroxide, shaking and centrifuging, removed very little porphyrin by adsorption. Moist precipitates were, therefore, prepared as follows:

(a) $0.5\ M$ - NaH_2PO_4 (2 vol.) was added to $0.5\ M$ - $CaCl_2$ (1 vol.) to give the soluble calcium dihydrogenphosphate according to the system $2(NaH_2PO_4) + CaCl_2 \rightarrow Ca(H_2PO_4)_2 + 2NaCl$. To this was added uroporphyrin solution and then N - $NaOH$ (1 vol.). Very little porphyrin was adsorbed by the precipitate which formed, $Ca(H_2PO_4)_2 + NaOH \rightarrow CaHPO_4 + NaH_2PO_4 + H_2O$. Addition of $0.5\ M$ - $CaCl_2$ (2 vols.) caused further precipitation but again very little porphyrin was carried down.

(b) Commercial $CaHPO_4$ was suspended in water and sufficient N - HCl added to

cause solution. Uroporphyrin solution was now added followed by excess of N - $NaOH$. The voluminous gelatinous precipitate adsorbed very little porphyrin. A similar result was obtained when phosphoric acid was used instead of HCl to dissolve the $CaHPO_4$.

(c) To $0.5\ M$ - Na_2HPO_4 (1 vol.) was added uroporphyrin solution and then $0.5\ M$ - $CaCl_2$ (1 vol.). There was, in this case, complete adsorption on the precipitated $CaHPO_4$.

(d) The precipitation of tricalcium phosphate was attempted by addition to the uroporphyrin solution of $0.5\ M$ - NaH_2PO_4 (1 vol.) and $0.5\ M$ - $CaCl_2$ (1.5 vol.) followed by N - $NaOH$ (1 vol.) in accordance with the theoretical reactions $2(NaH_2PO_4) + 3CaCl_2 \rightarrow Ca_3(PO_4)_2 + 2NaCl + 4HCl$ and $4HCl + 4NaOH \rightarrow 4NaCl + 4H_2O$. No porphyrin was adsorbed by the gelatinous precipitate.

(e) Reaction between $CaCl_2$ and Na_3PO_4 was brought about by addition in the uroporphyrin-containing solution of the calculated quantities of our reagents to give $Ca_3(PO_4)_2$ but the gelatinous precipitate carried with it very little porphyrin. It was observed, however, that precipitation of the phosphate was not quite complete as the addition of further quantity of $CaCl_2$ produced a small extra precipitate. *This adsorbed the whole of the porphyrin present* and the observation afforded the key to the entire problem. Wherever phosphate ions were in excess or could remain in the rather complex systems used, adsorption failed completely (e. g. b and d above) but when excess PO_4 was avoided (e. g. by addition of $Ca(OH)_2$ or $CaCl_2$ before alkalinization, adsorption occurred readily. The capacity

of sodium phosphate (over the whole pH range) to elute the adsorbed porphyrin was, therefore, tested with the result that in every case prompt elution occurred (see Table I).

Table I. *Elution of Adsorbed Uroporphyrin by 0.5 M - Sodium Dihydrogen Phosphate.*

Absorbent	System	Adsorption	Elution
Ca(OH)₂	CaCl₂ + NaOH	Complete	Complete
CaHPO₄	Na₂HPO₄ + CaCl₂	"	"
Ca₃(PO₄)₂	Na₃PO₄ + CaCl₂	"	Nearly complete
Mg(OH)₂	MgSO₄ + NaOH	"	Ppt largely redissolved
CaCO₃	Urine + CaCO₃ + NaOH	Partial	Complete
Kieselguhr	Solid + urine + HCl	Complete	Complete but slow

We thus realised that the varying success attending the adsorption of urinary porphyrins upon the phosphate precipitate brought down by addition of alkali was attributable to the varying proportionality of $Ca^{..}$ to $PO_4^{...}$ ions. Complete adsorption could regularly be ensured by achieving an excess of the former. As there is some competition between other urinary constituents, including the dark pigment of porphyria urine, and porphyrin for adsorption, greater quantities of the precipitate are required when working with pathological urines than when using simple solutions of porphyrins. The only addition that need be made, however, is calcium chloride since any excess of the latter is precipitated as $Ca(OH)_2$ by the sodium hydroxide which follows and this precipitate adsorbs porphyrin as well as do the phosphates.

A rough calculation shows that an addition of 3 mg CaCl₂/ml of a urine assumed

Table II. *Relation between Amount of CaCl₂ Added to Pathological Urine and Efficiency of Adsorption* (2.5 ml urine + x ml CaCl₂ 3 % (w/v) + N - NaOH to give final concn. of 0.5 N).

	CaCl₂ added. (ml. of 3% (w/v) soln.)	Uroporphyrin recovered from precipitate (mg/l urine).
Specimen A	0.5	8.65
	1.0	12.13
	1.5	12.18
	2.0	12.25
Specimen B	0.25	7.95
	0.25	7.99
	1.0	13.00
	2.0	13.07

to be low in Ca and high in P should suffice to combine with all $PO_4^{...}$ but for complete adsorption from pathological urines greater quantities than this are needed as is evident from Table II. For these experiments we used specimens of acute porphyria urine which were nearly black in colour (pretreatment with acid to convert porphobilinogen to porphyrin is described later) and contained approximately 13 mg of uroporphyrin/1. The precipitate carrying the porphyrin may be washed on the centrifuge once with 0.1 N - NaOH and once with water. Spectrophotometry demonstrated that no porphyrin was lost into the washings and that nothing was to be gained by more prolonged washing.

Spectrophotometric determination of the adsorbed porphyrin.

To recover the porphyrin, acid could be added to dissolve the precipitate and its adsorbed material, alternatively, the porphyrin could be eluted by repeated washing with sodium phosphate solution or with methanol

containing 1 % (w/v) H_2SO_4. Little was gained in the way of purity by the latter procedures which were more time-consuming. A solution of the precipitate of known volume was, therefore, made in 0.5 N-HCl and filtered from any debris present. The density was measured in the spectrophotometer at 430 mμ, 380 mμ and at the peak of the Soret band at 405 mμ (determinations of density at 406, 405, 404 and 403 mμ were made to ensure selection of the maximum). Application of the correction formula $p_{405} = \dfrac{2 D_{405} - (D_{430} + D_{380})}{1.844}$ (Rimington & Sveinsson forthcoming publication) then afforded a value for the absorption due to the uroporphyrin present. The value of $E_{1\ cm}^{1\ \%}$ for uroporphyrin was taken as 5000 (Rimington & Sveinsson). 0.5 N-HCl was selected as the solvent for the porphyrin as a result of trials in which the Soret band of pure uroporphyrin III was measured in acids of various strengths (Table III).

Table III. *Effect of Concentration of HCl on Intensity of Soret Band of Uroporphyrin III (1.6 μg/ml).*

Normality of Acid	Density (1 cm) at 405 mμ
0 05	0.70
0 1	0 78
0 2	0.80
0 5	0 70

The adsorption technique will generally be employed for the determination of total uroporphyrin in urines and for this purpose coproporphyrin is not likely to introduce any error. Not only is it usually present in porphyria urines in relatively much smaller amount but the Soret max. (401 mμ) is also so far removed from that of uroporphyrin that any contribution of the smaller quantity at 405 mμ can be ignored. The method is, however, capable of being used for the adsorption and recovery of coproporphyrin from urine but in this case 0.1 N-HCl was found to be the most satisfactory solvent for spectrophotometric determination.

As a routine procedure for porphyria urines, we add 1 ml of 3 % (w/v) $CaCl_2$ to each ml of urine taken for porphyrin determination. In earlier experiments we used a saturated (approx. 60 % (w/v)) solution of $CaCl_2$ of which 0.1 ml was added/ml urine and many recovery experiments were done employing this technique but we are satisfied that equally good results are obtainable with the smaller quantity. The supernatant from the adsorption should, however, be yellow-brown in colour and free from the dark reddish pigments which accompany uroporphyrin in porphyria urines.

Recovery of uroporphyrins I and III added to normal urine.

As an example of our standardized technique, the following experiment may be described in full. A solution was prepared of uroporphyrin III in 0.1 N-NaOH of approx. concentration 100 μg/ml, a 1/50 dilution having E 1 cm $= 0.840$. To 15 ml centrifuge tubes were added the following reagents:

	Tube 1	Tube 2	Tube 3	Tube 4
Urine (ml)	2	2	2	2
Uroporphyrin soln. (ml) .	1	0 5	0.1	0
Water (ml) . . .	0	0 5	0 9	1
3 % (w/v) $CaCl_2$ (ml) .	2	2	2	2
N-NaOH (ml)	5	5	5	5

The contents were well mixed and after about 10 min. centrifuged then washed by stirring and centrifuging with 0.1 N - NaOH and finally with water. 0.5 N - HCl was added to dissolve the precipitates and volumes brought to 50, 25, 10 and 10 ml respectively. After filtration, densities were measured on the Beckman spectrophotometer and recoveries calculated using the measured blank for correction (Table IV).

T a b l e IV. *Spectrophotometric Readings and Recoveries of Uroporphyrin III Added to Normal Urine.*

	No. 1	No. 2	No. 3	No. 4	No. 4 dild. 1/2.5	No. 4 dild. 1,5
Density at 404 mµ .	0.790	0.800	0.480	0.103	0.035	0.020
Calc. reco·very (%)	96.3	95.6	94.3			

T a b l e V. *Collected Results of Recovery Experiments after Addition of Uroporphyrins I or III or Coproporphyrin to Normal Urine.*

Porphyrin added to sample	Amount (µg)	Recovery (%)
Uroporphyrin III	100	98
	50	97
	10	96
	100	87
	50	103
	10	108
	100	94
	50	97
	10	103
		Mean 98.1
Uroporphyrin I	100	102
	50	120
	10	101
Coproporphyrin I	15	77
	10	73
	5	63

The acid solutions were stable for at least 24 hr., the longest period tested. In Table V we list the recoveries obtained from normal urine in a series of such experiments.

Recovery of uroporphyrin III added to pathological urines.

Owing to the fact that pathological urines may contain large quantities of pigments such as urobilin and porphobilin which might be adsorbed with the porphyrin, it became necessary to investigate the recoveries also from such pathological specimens. We chose an acute porphyria urine from which all the uroporphyrin had been removed by precipitation at the isoelectric point during prolonged storage in the ice chest. This specimen was a deep mahogany-red in colour due mainly to porphobilin.

Additions of 100, 80, 50, 40, 16 and 10 µg of uroporphyrin III were made to 2 ml lots of the fluid and the experiment conducted as for normal urine, one lot without addition of porphyrin serving as a directly measurable blank. The results were as follows (Table VI). It will be seen that recoveries were good; the use of the correction formula (Rimington & Sveinsson)

T a b l e VI. *Recovery of Uroporphyrin III Added to Deporphyrinized Acute Porphyria Urine.*

Uroporphyrin added (µg)	Recovery (%)
100	90
80	97
50	96
40	96
16	91
10	88
Mean	93.0

Table VII. *Uroporphyrin Content of a Series of Urines from a Case of Acute Porphyria.*

	1	2	3	4	5	6	7
Density at 405 mμ	0.624	0.788	0.226	0.415	0.471	0.470	0.195
Density at 430 mμ	0.154	0.196	0.050	0.098	0.110	0.102	0 068
Density at 380 mμ	0.227	0.280	0.075	0.138	0.156	0.150	0.094
Corrected value P_{405}	0.4701	0.5966	0.1770	0.3221	0.3665	0.3731	0.1236
Uroporphyrin (mg/l)	9.40	11.93	3.54	6.44	7.33	7.46	2.47
Uroporphyrin estimated by direct spectrophotometry of urine and application of correction formula (mg/l)	9.91	12.36	3.88	6.98	7.98	8.28	2.71
"Recovery" (%)	95	97	91	92	92	90	91

gave results agreeing closely with those obtained by subtraction of a directly determined black. Spectrophotometric examination of the diluted urine showed that the pigment it contained (porphobilin) exhibited no absorption bands over the region 400—550 mμ.

Application of the method to acute porphyria urines containing unknown quantities of uroporphyrin.

The recovery of different amounts of uroporphyrins I and III added to either normal or deeply-coloured pathological urines having been proved satisfactory, we proceeded to analysis of urines from cases of acute porphyria, using the formula based on the optical densities at 430, 380 and the Soret maximum at 405 mμ (see Rimington & Sveinsson) to correct for extraneous material contaminating the final acid solution of the calcium phosphate precipitate.

Pre-treatment of the urine. Waldenström (1937) has shown that freshly-passed urine from cases of acute porphyria contains porphobilinogen, a precursor which is only slowly converted to uroporphyrin and porphobilin or more quickly by warming in acid solution. It is customary to add hydro-chloric acid to a final concentration of ca. 0.25 N in the specimen under investigation and to leave it for 24 hr. or to heat for 10 min. in the boiling water bath. Extending the heating to 20 min. does not, in our experience, affect the final result. A study of methods of pretreatment will form the subject of a separate communication.

Adsorption, washing and resolution of the precipitate were carried out as described for normal urine. Usually 1 ml quantities of the pre-treated urine were taken for analysis, 1 ml of 3 % (w/v) $CaCl_2$ added then 2 ml of N - NaOH. After washing with 0.1 N - NaOH and water the precipitate was redissolved in 10 ml of 0.5 N - HCl, the solution filtered and examined spectrophotometrically.

Results. Some check on the values for uroporphyrin afforded by urines containing relatively large quantities could be obtained by examining spectrophotometrically a dilution of the urine made directly in 0.5 N -HCl and applying the correction formula. Results of analyses of consecutive daily specimens from a case are given in Table VII together with figures obtained by direct spectrophotometry. Examination of supernatants

(acidified) after adsorption showed that no detectable porphyrin remained unadsorbed.

Satisfactory results were also obtained when different quantities of urine were taken for analysis (see Table VIII).

Table VIII. *Results of Analyses of Different Quantities of the same Urine.* In each case, 2 ml of 3 % (w/v) $CaCl_2$ were added followed by N - NaOH to a final concentration of 0.5 N.

Vol. urine used (ml)	Uroporphyrin found (mg/l)
3.0	13.68
2.5	13.52
2.0	13.78
1.0	13.60
Uroporphyrin by direct spectrophotometry on dil. urine	13.38

Separate determination of uroporphyrins I and III.

The porphyrin fraction which Waldenström, Fink & Hoerbürger (1935) designated uroporphyrin III is extractable by ethyl acetate from aqueous solution within the narrow range of pH 3.0-3.2 whereas the series I isomer is stated not to be so extracted. Our calcium phosphate adsorption procedure measures both uroporphyrins I and III but by carrying out a separate determination of the ethyl acetate-soluble fraction, differential determination is possible. In the first instance, we took samples (10 ml) of the pre-treated porphyria urines, adjusted these to pH 3.1 (glass electrode) then, using citrate buffer of the same pH to rinse the electrodes, adjusted the volume to 15 ml. Samples (2 ml) were extracted by shaking twice with ethyl acetate (10 ml), the combined extracts washed once with a little citrate buffer pH 3.1 and the porphyrin then passed into 0.5 N - HCl by three successive shakings (final vol. 10 ml). Spectrophotometric determinations on these solutions gave figures for the directly extractable porphyrin.

In applying the same process to the redissolved calcium phosphate precipitate, derived from 1 or 2 ml of urine according to total uroporphyrin content, these were adjusted to pH 3.1 (glass electrode) buffer added and the mixtures shaken twice for 3—4 min. each time with 5 vol. ethyl acetate (10 vol. in all). The combined extracts were shaken with 0.5 N - HCl using 4 lots and adjusting finally to 10 ml for spectrophotometric determination. Control experiments with stock uroporphyrin III showed that under these conditions 92—100 % was recoverable. Despite much effort, greater precision could not be achieved. Agreement was satisfactory between the percentage found to be ethyl acetate soluble of the total uroporphyrin in the adsorbate and in the urine itself. The results indicated that in only two specimens

Table IX. *Uroporphyrin III Content, Determined by Ethyl Acetate Extraction at pH 3.0—3.2, of a Series of Urines from a Case of Acute Porphyria and of the Calcium Phosphate Adsorbate Prepared from these.*

Total uroporphyrin	Uroporphyrin III by extraction	
	of adsorbate (% of total)	of urine (% of total)
11.02	91.9	99.7
3.69	99.6	100.0
6.84	84.8	82.2
7.38	83.3	87.4
7.53	98.5	107.2
2.58	99.6	97.6

from the patient was the difference between total and ethyl acetate soluble porphyrin appreciably greater than the experimental error (Table IX).

Application of the method to congenital porphyria urine.

The specimen of urine from a human case of congenital porphyria had a deep burgundy red colour. It was very rich in porphyrin and had a pH of 6.0 but was rather low in phosphate, containing 0.13 g P_2O_5/100 ml.

In preliminary experiments quantities of 0.25 and 0.5 ml were diluted with water to 2 ml, 3 % $CaCl_2$ solution added (1 ml.) then N - NaOH (3 ml) but adsorption upon the small precipitate was incomplete. Loss of porphyrin could be obviated either by adding larger quantities of $CaCl_2$ (e. g. 4.0 ml of 3 % solution to 0.5 ml of urine) or, preferably, by increasing the phosphate by addition of 0.5 ml of 0.2 M - Na_3PO_4 to 0.5 ml of urine, then precipitating by 4 ml of 3 % $CaCl_2$ solution followed by 5 ml of N - NaOH.

A series of 8 determinations performed in this manner (with slight variations in the quantity of reagents used) afforded a uroporphyrin content of 38.94 mg/l. S. D. ± 1.47.

DISCUSSION

The provision of a rapid and accurate method for the determination of uroporphyrin in pathological urines should greatly facilitate the study of the porphyrias. The method here described is very convenient and

is applicable even to very darkly coloured urines. Sufficient calcium should always be present to ensure the precipitation of all phosphate ions but if the uroporphyrin content of the specimen being examined is very high it is advisable also to increase the quantity of the precipitate by the prior addition of a little sodium phosphate.

The relation between the solid calcium phosphate phase, PO_4 ions and porphyrin in solution is an interesting one recalling the similar behaviour of fluoresceine in the titration of soluble chlorides by a silver salt. The precipitate adsorbs chloride ions and these are, only removed when a slight excess of silver is present. If fluoresceine is also present, its anion competes unsuccessfully with the chloride ion for adsorption and only achieves its object when the last trace of chloride has been precipitated.

The observation that PO_4 inhibits the adsorption of uroporphyrin by calcium phosphate offers an explanation of the fact that in congenital porphyria or after the injection of uroporphyrin into animals, it is only at sites of active calcification (young bone or fracture callus) that deposition of the porphyrin occurs. In these regions the solubility product for calcium phosphate must be exceeded by an excess of either ion.

According to Robinson's scheme of calcification, a local increase in phosphate ions is brought about by the action of bone phosphatase upon phosphoric· esters but others have stressed the importance of a proteolysis liberating proteinbound calcium as the final incident leading to the mineralization of the tissue.

SUMMARY

1. The adsorption of porphyrins by calcium phosphate precipitates has been studied.

2. It is found that adsorption is very slight so long as free phosphate ions remain in the solution but when all PO_4''' is precipitated the adsorption of porphyrin is quantitative. Similarly, solutions of soluble phosphates will elute porphyrins from calcium phosphate precipitates on which they are adsorbed. This behaviour is compared with that of fluoresceine used as an adsorption indicator in the titration of soluble chlorides by silver salts.

3. A method based upon these principles, has been devised for the quantitative determination of uroporphyrin isomers in pathological urines and has been shown to give satisfactory results in recovery experiments with normal and pathological urines and in determinations carried out on urines from cases of acute and congenital porphyria.

4. The proportion of ethyl acetate soluble and insoluble uroporphyrin may be determined in the concentrate obtained by this method.

5. Since total urinary coproporphyrin is determinable by ether extraction and the proportion of the two isomers by application of published methods, a complete porphyrin analysis of pathological urine becomes possible.

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QUALITATIVE ANALYSIS OF THE PORPHYRINS
BY PARTITION CHROMATOGRAPHY

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For the biochemical study of clinical materials, it is highly desirable to have a method for detecting qualitatively very small quantities of porphyrins and for identifying the individual constituents of mixtures of these pigments. The method to be described, which is based upon partition chromatography, has the advantage that quantities as little as half a microgram of porphyrin can be identified with certainty.

Partition chromatography differs from the well known adsorption chromatography introduced by Tswett in that the separation is brought about by the difference in partition of the substances between two liquid phases. Any two liquids can theoretically be used but so far the only published systems have had an aqueous phase as one component. The stationary phase, usually the aqueous one, is supported either on a column of some water-absorbing substance such as silica gel, starch grains or cellulose pulp or more conveniently still, when dealing with very small quantities, on strips of filter paper. The latter method was introduced by Consden, Gordon and Martin (1944) and it is this method which we have used.

METHODS

The technique is as follows: the material to be analysed is placed on a marked spot about 6 cm from one end of a long strip (ca. 35 × 6 cm) of ordinary filter paper. Whatman No. 1 is a suitable quality. The paper is supported so that one end dips into a small glass trough (Fig. 1). The non-aqueous solvent chosen as the second phase is first saturated with water by shaking in a separating funnel at the temperature at which the chromatogram is to be run, then placed in the trough and allowed to creep by capillarity down the paper. The whole apparatus is enclosed in an airtight box or glass jar containing small beakers of water and the solvent respectively, so that the atmosphere is saturated with respect to both phases, and precautions are taken to prevent the temperature of the system from altering. Each substance is distributed according to its partition coefficient between the water, bound in the fibres of the filter paper, and the second solvent. The component whose partition in favour of the solvent is greatest will travel farthest from the starting point and conversely. Thus one obtains a series of discrete spots down the filter paper. The ratio of the *distance travelled by the solute to the distance travelled by the solvent is termed the Rf value.* Thus, if the Rf of a substance is 1, it travels with the solvent front, partition being completely in favour of the non-aqueous phase.
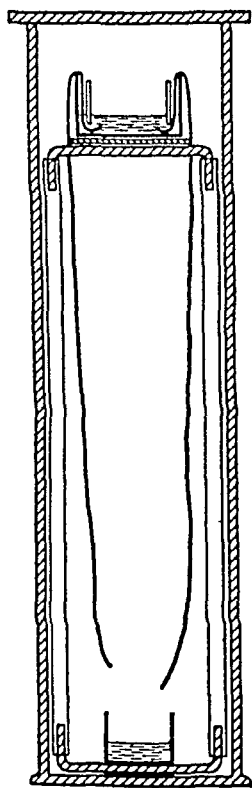
Fig. 1. Diagram of apparatus used for partition chromatography.

The fact that the strip of paper acts as a support for the two solvents and does not adsorb the *solute* has been proved by Consden, Gordon and Martin (1944) in the case of the amino acids by calculating expected Rf values from their known partition coefficients and the percentages of water and solvent on the paper.

When using an aqueous phase, the solute must have a hydrophylic group. The hydrophylic tendency of such groups can often be enhanced or diminished by varying the atmosphere surrounding the chromatogram. For example, in ammonia vapour a carboxyl group is converted to ammonium salt, in hydrogen chloride, a basic group to the hydrochloride. Saturation of the atmoshpere may be achieved by inserting in the box a beaker containing concentrated aqueous ammonia or hydrochloric acid.

Thus, according to conditions, substances can be made to move as neutral molecules, anions or cations. Even neutral inorganic salts such as sodium chloride, in phenol-water-ammonia systems are split up, the ions running at different rates and so becoming separated spatially, as may be shown by appropriate tests (R. G. Westall, 1948). Clean partition of the porphyrins is interfered with by inorganic salts such as NaCl and it is possible that the occurence of such ionic separation may be responsible.

Since porphyrins are ampholytes, the possibilities exist of treating them as anions, as cations, or after esterification, as weak bases. In the latter case they retain very little hydrophylic tendency and we have not succeeded in finding a suitable system to separate them in which one of the phases is aqueous.

The solvent system which has been found to be most satisfactory in the case of the free porphyrins is the lutidine-water system in presence of ammonia vapour. We use a commercial mixture of the two lutidines, 2:4 and 2:5 dimethyl pyridine. With this system at constant temperature, mixtures of porphyrins are clearly separated. The positions of the spots can either be seen by their ultra-violet fluorescence or in the case of larger quantities by their red colour in ordinary light: moreover the pigment in individual spots can be identified by its absorption spectrum observed by merely holding the paper in front of a spectrometer with suitable illumination. This procedure is most useful in the case of metal complexes — which do not fluoresce in in U. V. light.

RESULTS

Table I records the Rf values found in the lutidine-water-ammonia system for a number of porphyrins and Fig. 2 is a photograph of a chromatogram obtained from an artificial mixture of porphyrins. It will be seen that a relation exists between the Rf and the number of carboxyl groups in the molecule. This relationship is illutsrated graphically in Fig. 3.
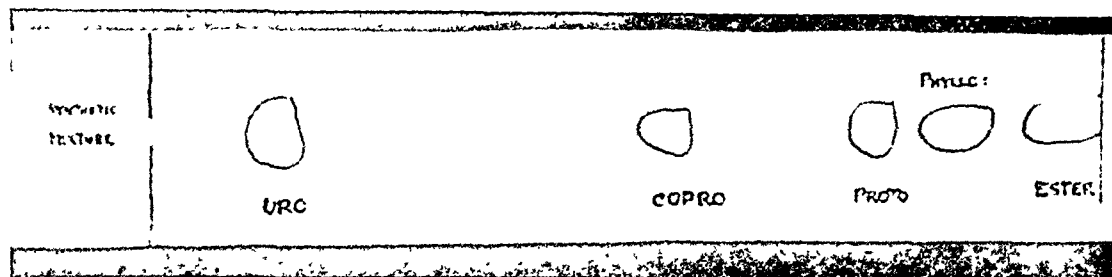
Fig. 2. Partition chromatogram obtained with an artifical mixture of porphyrins.

Table I. *Rf values (at 19° C) of por-phyrins in lutidine in atmosphere of ammonia vapour.*

Porphyrin	No. of carboxyl groups	Rf value
Uroporphyrin	8	0.3
Coproporphyrin	4	o 6
Protoporphyrin	2	o 8
Deuteroporphyrin	2	0.8
Mesoporphyrin	2	o 8
Haematoporphyrin	2	0.8
Phylloerythrin	1	o 9
Monazaprotoporphyrin..	2	o 8
All porphyrin esters...	o	1.0

It would appear that the group in the porphyrins having the greatest lyophylic property is the carboxyl group and it is seen that the Rf value is inversely proportional to the number of anionic groups. If all the carboxyl groups are esterified the lyophylic properties are removed and the esters are found to have an Rf of 1. The Nitrogen atoms of the heterocyclic rings would appear to have little effective hydrophylic character. Some of the metal complexes, in which these basic groups are no longer free have been investigated by the lutidine chromatogram. In the case of the copper complexes, the Rf values are the same as those of the free porphyrins. Both the iron and cobalt salts have an Rf 0.1 less indicating the presence of an additional acidic group.

It is of interest that Morrison and Williams (1941) have found from pH titration curves that haemin possesses three titratable acidic groups. These are depicted as follows.

Titratable Acidic Groups in Haemin.

$$(HOOC)_2 \cdot Por \cdot FeCl +$$
$$+ \; 3\,NaOH \rightarrow (NaOOC)_2$$
$$Por \cdot FeOH + NaCl + 2\,H_2O$$
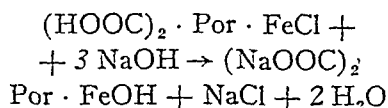
(Morrison and Williams, J. biol. Chem: *137*, 461)

Rf of Haemin $= 0.7 = 3$ Acidic Groups.
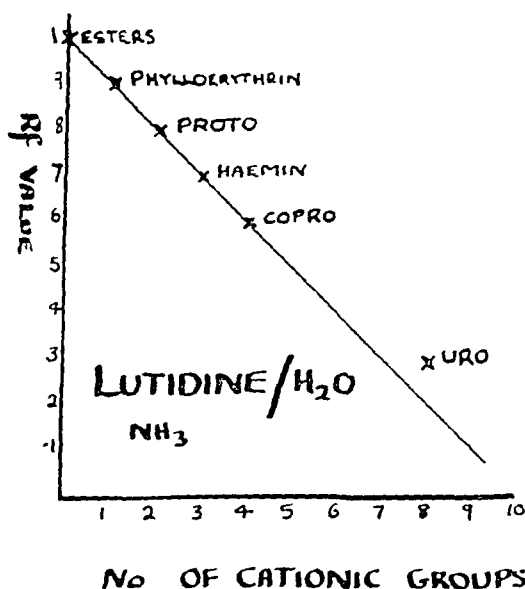
Fig. 3. Graphical representation of relationship between number of cationic groups and Rf values in lutidine/water system.

Many other solvents have been tried but the majority of these either did not move the porphyrin, or the porphyrin ran all the way with the solvent indicating complete solubility in one or other of the phases and no partition. However, the systems phenol/-water, methylethyl ketone/water, *m*-toluidine/glycerol and pyridine/aqueous potassium carbonate, have resolved the porphyrins to a limited extent. Table II shows solvent systems used.

Attempts were made to separate the isomers Uroporphyrin I and Uroporphyrin III using phenol/aqueous phosphate buffer solution at controlled pH values around the isoelectric point of the isomers. These attempts were not successful but we found that the specimens of Uroporphyrin used gave multiple spots. This would indicate a heterogeneity which we think might possibly be attributable to bond isomerism of the molecule.

T a b l e II. *Rf values obtained for free porphyrins in other solvent systems.*

S = sample streaked   No. = Rf value
O = sample did not move   I.S. = Insufficient separation

Solvent system	Uro	Copro	Meso	Proto	Deutero	Haemato	
Phenol/water	0.1	0.7	0.9	0.9	0.9	0.9	S.
Phenol/water (HCl atmos.)	0.7	1.0	1.0	1.0	1.0	1.0	I.S.
Phenol/water (CH$_3$COOH atmos.)...........	0.6	1.0	1.0	1.0	1.0	1.0	I.S.
Phenol/water (NH$_3$ atmos.)	0.4	0.6	0.8	0.8	0.8	0.8	S.
o-Cresol/water	0.1	0.4	0.8	0.8	0.8	0.8	S.
o-Cresol/water (CH$_3$COOH atmos.)	0.7	1.0	1.0	1.0	1.0	1.0	I S.
Butyl alcohol/water (CH$_3$COOH atmos.)......	0.2	0.8	0.8	0.8	0.8	0.8	S.
Butyl alcohol/water (HCl atmos.)	0.2	0.8	0.8	0.8	0.8	0.8	S.
Amyl alcohol/water (CH$_3$COOH atmos.)	0.1	0.5	0.8	0.8	0.8	0.8	S.
Amyl alcohol/water (HCl atmos.)	0.1	0.5	0.8	0.8	0.8	0.8	S.
Benzyl alcohol/water (CH$_3$COOH atmos.)	0.1	1.0	1.0	1.0	1.0	1.0	S.
Quinoline/water	0	1.0	1.0	1.0	1.0	1.0	I.S.
Quinoline/water (NH$_3$ atmos.)	0	0	0.1	0.1	0.1	0 1	S.
Cyclohexanol/water	0	0	0.5	0.5	0.5	0.5	S.
Glycol monoethyl ether acetate/water	0.5	1.0	1.0	1.0	1.0	1.0	S.
Diamylamine/water	0	0	1.0	1.0	1.0	1.0	S.
Triethylamine/water	0	0	1.0	1.0	1.0	1.0	S.
Methyl ethyl ketone/water	0	1.0	1.0	1.0	1.0	1.0	I.S.
Methyl ethyl ketone/water (HCl atmos)	0.5	0 5	0.8	0.8	0.8	0.8	I.S.
Methyl ethyl ketone/aqueous buffer pH 3	0.6	0.9	0.9	0.95	0.9	0.85	I.S.
m-Toluidine/glycerol	0.2	0 25	1.0	1.0 .	1.0	1.0	I.S.
Pyridine/aqueous K$_2$CO$_3$	0	0.4	1.0	1.0	1.0	1.0	
Aqueous K$_2$CO$_3$/pyridine	0.8	0.3	0.1	0.1	0.1	0.1	

The following solvent systems were found to produce no movement of the porphyrins —

Butyl alcohol/water	Diethyl ether/water	Aniline/water
Amyl alcohol/water	Butyl ether/water	Cyclohexane/water
Benzyl alcohol/water	Methyl phenyl ether/water	Nitrobenzene/water
Ethyl acetate/water	Chloroform/water	Benzene/water
Methyl salicylate/water	Benzyl benzoate/water	Xylene/water
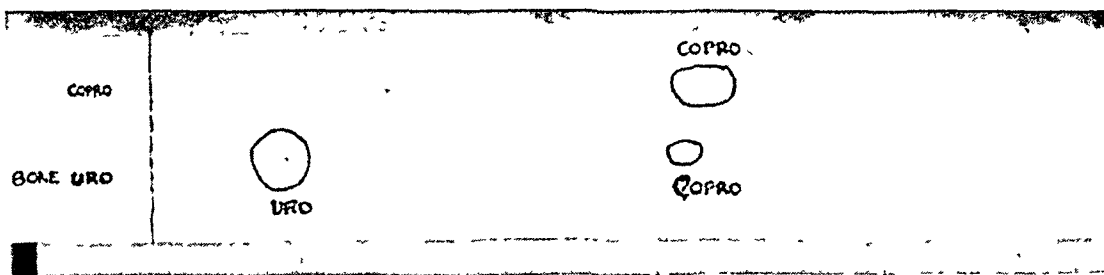
Fig. 4. Partition chromatogram of uroporphyrin preparation seen still to contain some coproporphyrin.

APPLICATIONS

This method of separation of the porphyrins can be employed for many purposes — as follows.

(a) As a test of homogeneity. We have thus identified small quantities of coproporphyrin present in supposedly pure preparations of uroporphyrin (Fig. 4).

In designing this method, we have had particularly in view its application to the study of the Waldenström uroporphyrin, concerning which so much controversy now exists. This ethyl acetate-soluble fraction from acute porphyria urines would appear under certain conditions to behave as an entity yet Watson and others claim to have separated it chromatographically on calcium carbonate columns and to have obtained both coproporphyrins I and III after decarboxylation (Grinstein, Schwartz and Watson, 1945).

It was hoped that partition chromatography would supply independent evidence for the existence or otherwise in the Waldenström ester of the heptacarboxylic porphyrin described by Watson. Unfortunately the complex behaviour of the uroporphyrins in paper chromatography renders difficult a decisive interpretation but we may say that we have not as yet seen any indication of the presence of a heptacarboxylic porphyrin.

(b) For the identification of constituents in natural mixtures. Of particular interest in this connection was the examination of the total porphyrin from *normal* urine. *We have found it to contain a small quantity of uroporphyrin in addition to coproporphyrin.* Although uroporphyrin occurs in the urine of other animals and in human cases of acute and congenital porphyria, its presence in normal human urine has been much disputed. Fischer and Zerweck (1924) found traces, in 100 litres, of an ether-insoluble porphyrin, one absorption band only of which could be measured, but no more exact characterization has been forthcoming and other workers have even denied the presence in normal urine of any ether-insoluble porphyrins. We worked with the total porphyrin from 4.5 litres. This was concentrated by adsorption upon calcium phosphate, purified on a column of talc developed with dilute aqueous ammonia, which removed much extraneous pigment, and then eluted
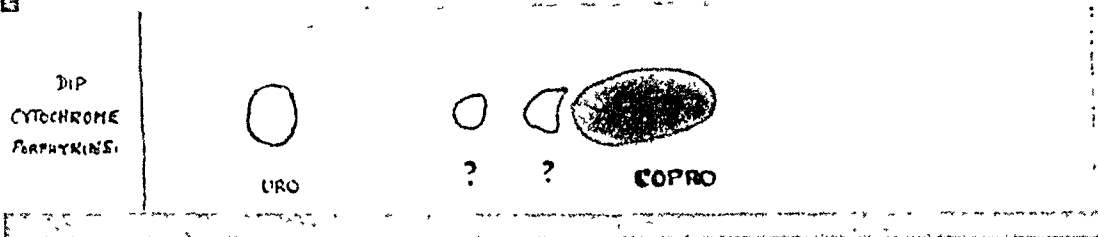
Fig. 5. Partition chromatogram of porphyrins produced by *Corynebacterium diphtheriae*.

by concentrated ammonia. The partition chromatogram was made with lutidine-water-ammonia in the usual way.

As a second example of analysis of a complex mixture, the examination may be described of the porphyrins produced by *Coryne-bacterium diphtheriae* grown in a synthetic medium. Gray and Holt (1947) have described the isolation from this source of coproporphyrin III together with uroporphyrin I and our results confirm these findings and indicate the presence, in addition of two other porphyrins which, judged from their positions in the chromatogram, should possess carboxyl groups intermediate in number between 4 and 8 (Fig. 5).

Dr. Comfort at the London Hospital has been working on shell pigments with special reference to the porphyrins they contain. We have helped him to establish the identity of copro- and uro-porphyrins occurring together in several shells including those of *Pteria radiata*. This genus was examined by Fischer and Jordan (1930) and Fischer and Haarer (1932), the former workers describing a pentacarboxylic porphyrin which they termed "conchopor-phyrin" in *Pteria radiata*, although Fischer and Haarer found only uro- and coproporphyrins in *Pteria vulgaris*. Through the

kindness of Professor Waldenström we have been able to examine a fraction (M.P. 270°) obtained by Fischer during the isolation of conchoporphyrin. In it we find only uropor-phyrin and coproporphyrin.

(c) Examination of pathological speci-mens. Pathological urines have been dia-lysed in cellophane membranes to remove sodium chloride and other interfering sub-stances. The porphyrins are retained on the inner surface of the membranes from which they may be removed for analysis. In cases of Congenital and Acute Porphyria the presence has been confirmed of copro- and uroporphyrins together with an unindenti-fied porphyrin (Fig. 6).

CONGENITAL PORPHYRIA
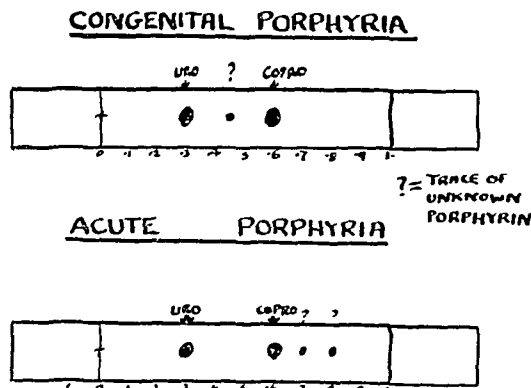
Fig. 6. Diagram of Partition Chromatograms obtained with congenital and acute porphyria urines.

This method should find use in the study of metabolic disorders which cause an increase in porphyrin excretion such as lead poisoning, diseases caused by hepatic lesions, etc.

(d) The structure of new pigments. This technique is obviously applicable to the study of the constitution of new pigments of unknown structure.

DISCUSSION

Although it has not yet proved possible to elaborate a two-dimensional system for the partition chromatography of the porphyrins the unidimensional technique described above is capable of separating porphyrins into classes according to the number of carboxyl groups they contain. In the analysis of complex biological mixtures this is of great assistance and the method is applicable to as little as 0.5 μg of pigment.

The technique demands only simple apparatus and requires attention only in the initial and final stages so we believe it should prove suitable for adoption in the clinical chemical laboratory.

SUMMARY

1. The technique of paper partition chromatography has been applied to the examination of the porphyrins.

2. The most suitable solvent so far found is lutidine (mixture of 2 : 4 and 2 : 5 dimethyl pyridines) in the presence of ammonia vapour.

3. Under these conditions separation occurs according to the number of carboxyl groups in the molecule.

4. Examples are given of the application of the technique to the examination of natural materials and patholoigcal specimens.

ACKNOWLEDGEMENTS

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ELECTROMETRIC CHLORIDE TITRATION
IN BIOLOGICAL MATERIAL

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The electrometric determination of the end point in a titration has definite advantages over methods with a color change. For one thing the electrometric end point is quite objective. Further the sample may be colored or turbid without interference. And finally electrometric titrations are conveniently adapted to the micro scale.

Estimation of chloride ions is an especially favorable application of electrometric titrations. In spite of this the electrometric determination of chloride ions does not seem to be a common method in hospital laboratories. This may be due to the lack of a suitable and inexpensive apparatus. The present paper describes such a unit which has served well for over 2 years in this laboratory. It was built from parts made by domestic manufacturers and with limited workshop facilities.

DESCRIPTION OF APPARATUS

This is composed of a reversible chemical cell (Ag, sample $\parallel$ 1 N K_2SO_4, Hg_2SO_4, Hg), a simple potentiometer arrangement in order to compensate for the end point potential from the cell, a press-button switch, and a null point instrument. The arrange-

ment of these parts and some other details emerge from the figure.

As null point indicator a microampère-meter, 10—0—10 μA, 4000 ohms internal resistance, is used.[1] Of course a galvanometer with suitable properties will serve equally well, e. g. the Pot galvanometer (Cambridge Instrument Company) or pointer galvanometer No. 2310-c (Leeds and Northrup).

DIRECTIONS FOR USE

Protein containing samples (blood, serum, lymph etc.) are first deproteinized. Take 0.100 ml into 4.9 ml of distilled water. Add 2.0 ml of 1 N trichloroacetic acid, mix and centrifuge for about 5 minutes. Take 5.0 ml of clear supernatant into a small glass beaker (30 $\times$ 45 mm). Place the beaker on a platform so that the silver rod and the siphon dip into the solution. Add 0.005 N $AgNO_3$ from a micro burette in small portions. After each addition shake the beaker back and forth on the platform to insure complete mixing (the use of a mechanical glass stirrer is of course more comfort-

[1] Type VRR 11, manufactured by Ermi, Ulvsunda 1, Sweden.
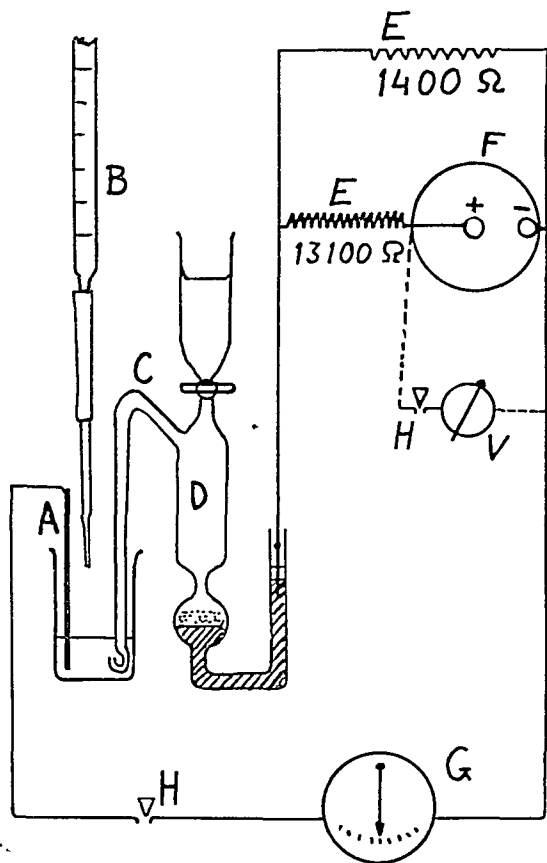
Fig. 1.

A silver rod 2 mm   G galvanometer (microammeter)
B microburette   H press-button switch
C syphon with   V voltmeter
   1 N K₂SO₄
D reference half cell, Hg/Hg₂SO₄, 1 N K₂SO₄
E resistances constituting potentiometer
F dry cell, 1 5 V

able, though not necessary) and close the electrical circuit for a moment. When the deflections of the microammeter are decreasing the end point is near, and the silver nitrate is now added drop by drop until the meter gives no deflection even at a long closing. The end point is sharp to a fraction of a drop. At the end of the titration a drop is let out from the siphon and the latter and the silver rod are washed with some

distilled water. Run duplicates on each sample. As the trichloroacetic acid usually contains some chloride a blank must be run every day. When not in use keep the open end of the siphon in a beaker with water.

Calculation: let a ml be the amount of $AgNO_3$ used for he sample and b ml for the blank, then, if, the $AgNO_3$ is c N, the sample contains

$$\frac{7 \cdot (a-b) \cdot c}{5 \cdot 0.1} = 14 \ (a-b) \cdot c \, N \, Cl'.$$

Samples which are protein free may be taken directly into the titration beaker with enough water, acidified with a few drops of dilute HNO_3, and titrated as just described. A blank must also be run.

CONTROL

The simplest way of control is to titrate different amounts of a standard solution. e. g. 0.5, 1.0 and 2.0 ml of a 0.0100 N KCl. After correction for the blank the values must be strictly proportional. If this is not so or if the endpoint is not sharp, the following controls are made: 1) check the potential of the dry cell. If less than 1.43 V, insert a new cell. 2) see that the springs in the switch are polished and give a good contact. 3) clean the silver rod by short immersion in hot strong nitric acid and wash with distilled water. — If these steps do not remedy the troubles, a regular titration curve must be made with a potentiometer that can be read from 0 to 300 mV. A change in the end point potential indicates a change in the mercurous sulfate electrode, which then must be replaced. However no indication of such changes has been found with the three units built by the author since 1945.

COMMENTS

The completely chloride-free reference electrode selected avoids the troubles with a chloride-free connection between the titration half cell and the reference half cell which is necessary in argentometry if the latter is a calomel electrode. With the reversible cell described above the end point potential for chloride titrations at room temperature was found to be —140 mV. The dry cell ususally has a potential of 1.47 to 1.45 V. Therefore the resistances in the potentiometer were selected so as to give —140 mV at 1.45 V. Then at the limits of 1.47 and 1.43 V the compensating potential varies between —142 and —138 mV. This gives identical titration values.

Due to its simplicity this apparatus with proper handling gives a very good reproducibility, the dispersion being only 0.9—0.4 % of the titration value for 7.5—30 micromols of Cl' (266—1065 μg Cl'). In clinical routine and with different operators the standard deviation is naturally somewhat higher. For one month (4 different operators) the coefficient of variation calculated from all duplicates on whole blood was 1.3—1.8 %. This is for most purposes sufficient, especially when the normal variations are large as in blood (85 ± 9 mM in whole blood [*Karlson & Norberg,* 1926], 100—107 mM in serum according to current textbooks) but also for spinal fluid where the normal value is extremely constant (123.1 ± 0.35 mM, *Karlström,* 1942) and thus already small deviations may be significant.

SUMMARY

The construction and use of a simple apparatus for the electrometric determination of chloride in biological fluids is described.

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THE RENAL BLOOD FLOW AND THE GLOMERULAR FILTRATION RATE IN CONGESTIVE HEART FAILURE AND SOME OTHER CLINICAL CONDITIONS

THE EFFECT OF EXERCISE AND HYPOXEMIA. A PRELIMINARY REPORT

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The renal blood flow, as determined by p-aminohippuric acid or diodrast clearance, *is receiving increasing attention.*

So far only a few reports dealing with the *renal blood flow in congestive heart failure* have appeared in the literature (Merrill 1946, Merrill and Cargill 1948, Mokotoff et al. 1948). These investigations show that the renal blood flow often is low in cardiac subjects. A corresponding reduction of the filtration rate also occurs, but not to the same extent as the renal blood flow. This means that the filtration fraction, i. e., the glomerular filtration rate in per cent of the renal blood flow, usually increases. According to Merrill there is no relation between the venous pressure and the renal blood flow. In congestive heart failure, however, there is a correlation between the cardiac output and the renal blood flow, which he thinks indicates that the reduction in renal blood flow is not due to venous congestion but to an inadequate cardiac output, i. e., a forward and not a backward failure.

In the publications mentioned practically no attempt is made to correlate the renal function and the clinical condition of the patient. Information concerning the exact anatomical and functional diagnosis, e. g., right-sided contra left-sided failure, is incomplete or missing.

Merrill and Cargill (1947) have examined the *effect of muscular exercise on the renal blood flow in cardiac subjects.* They differentiate between patients with a tendency to edema formation and those without, and have found a certain correlation between the edema on the one hand and the reduced renal blood flow and glomerular filtration rate on the other. In 6 out of 10 cardiac subjects they found during light exercise a comparatively marked reduction in glomerular filtration rate and also a fall in the renal blood flow. The information regarding clinical dates, however, is incomplete.

During *muscular exercise in normal persons* Merrill and Cargill, and later on Chapman et al. found a fall in the renal plasma flow and usually a reduction of the glomerular filtration rate.

The effect of exercise on the filtration rate as determined by creatinine clearance

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[1] Held in a somewhat more extensive form as a lecture at a meeting of the Norwegian Society of Internal Medicine on Oct 11th, 1948.

and urea clearance is known from previous publications, e. g., those by Grande Covian and Rehberg (1936). During exercise there is always a fall in the diuresis on the whole paralleling the intensity. As a result of light exercise there is no fall in the creatinine clearance, though a fall will occurr during heavier exercise. A fall in the urea clearance however, will be seen comparatively early. As soon as the exercise is interrupted, conditions rapidly return to normal.

A closer investigation of what actually takes place within the kidney in the various forms of heart disease, i. e., in different forms and stages of failure, seems justified. Moreover, the response of normal and cardiac subjects to exercise has not been sufficiently studied. Nor have any reports appeared on the effect of hypoxemia in normal and cardiac subjects.

Measurements of the cardiac output by accurate methods are of great interest in relation to problems concerning the renal blood flow. There is not always a direct correlation between these two values. Adrenine, ephedrine, etc. cause an increased cardiac output but a reduced renal blood flow, whereas the filtration rate on the whole remains unchanged (H. Ranges and St. E. Bradley 1943). Artificial fever causes an increased renal blood flow as well as an increased cardiac output. The latter, however, is not increased to the same extent, indicating a relatively more marked vasodilation in the kidneys than in the circulatory system as a whole (St. E. Bradley et al. 1945). When various conditions involving an increased cardiac output are investigated, nothing can be predicted about the renal blood flow

In our investigation of the renal blood flow we have included a study of normal individuals at rest, during exercise, and hypoxemia. We have also studied patients with various forms of heart disease and heart failure. Further, we have examined changes in renal blood flow and glomerular filtration rate in severe anemias and in the same patients following recovery. Finally, we have made an investigation into the relationship between the renal blood flow and basal metabolism by studying patients with thyreotoxicosis and the same patients when the basal metabolism had been reduced or brought back to normal. We do not know about previous reports on the relation between basal metabolism and renal blood flow. St. E. Bradley and G. P Bradley (1947) have shown that in patients with chronic anemia there is a reduction of the total renal blood flow, and that an increase occurs when the anemia improves. This is another example of the incomplete correlation between cardiac output and renal blood flow. Anemia regularly implies an increase in the cardiac output closely correlated to the reduction of hemoglobin percentage.

The renal blood flow is an important function and for this reason no doubt highly safeguarded. The blood which normally passes the kidneys constitutes a comparatively large part of the basal cardiac output (about one fourth). This enables us by simple means to obtain informations of a considerable percentage of the total blood stream as well as information regarding the effect of changes in the blood flow on the functions of a vital organ.

We have avoided the problems connected with hypertension as well as renal diseases. Such subjects were selected, who had a normal blood pressure (with a single exception) and who did not present clinical evidence of renal disease.

The validity of the PAH-method in heart diseases is discussed below.

METHODS

The technique has been extensively described by earlier writers. The experimental conditions, however, need further presentation as they to some extent can be said to be original.

Para-aminohippuric acid is determined in the blood after precipitation with 30 % trichloracetic acid and otherwise as described by Homer Smith et al. (1945). The colorimetric determinations of PAH, inulin and creatinine have been carried out by means of a Klett Sumerson's photoelectric colorimeter which we have found most satisfactory. PAH has not been available in Norway. We are, therefore, much indepted to Sharp and Dome, Philadelphia, who has supplied us with a number of ampoules of PAH so that we were able to complete our investigations. The reagent N-(1-naphtyl)-etylene-diamine-dihydrochloride has been syntezised by Dr. L. Eldjarn and Dr. Phil. Bailii Nilssen, Oslo. Not until recently, i. e., after one year the reagent has been obtained from U. S. A.

Inulin has been determined in the blood after precipitation with Somogyi's solution and otherwise by the diphenyl-amine method (Goldring and Chasis) as described by Bertil Josephson and Anna-Stina Godin (1943). Certain details will be considered in a later publication. Inulin was supplied through the courtesy of Astra, Södertälje, Sweden.

Endogenous "Creatinine" is that or those chromogenous substances which are responsible for the color in Jaffe's picrate reaction. Endogenous "creatinine" has been determined in the same filtrate as inulin (Folin). The precipitation with Somogyi's solution gives a plasma dilution 1 to 10. With the extremely low normal concentration of "creatinine" in the blood a dilution of 1 to 4 or 1 to 5 would probably have been better.

Urea was determined in the Somogyi filtrate by Van Slyke's manometric hypobromite method.

EXPERIMENTAL CONDITIONS

The standarized technique described by H. Smith et al. (1946) was used for clearance measurements. As to the sustaining infusion we have adopted the use of 8 ml instead of 10 ml PAH. The PAH dose used for the priming infusion has also been somewhat reduced, as the blood level is otherwise apt to be a little too high if the renal blood flow is impaired. All urine collections were carried out with a cathcter. The bladder was washed out with tap water and air introduced, and urine expressed with pressure over the bladder.

The period between the priming infusion and first urine collection was originally 20 minutes, but was later on changed to 30 minutes. Otherwise urine collections were made at interval of 20 minutes or less. An adequate diuresis was secured by giving the patient an appropriate amount of water before the test period.

Blood samples were usually drawn in the middle of the clearance period. Two clearance periods were commonly used, sometimes several periods, and the figures shown represent average figures.

The hypoxemia tests have been done as follows. After one or several 20 minutes "rest" periodes the subject has inhaled 9.5 % O_2 during one or several periodes, in the same way as we do it in electrocardiographic hypoxemia tests. In a final clearance period the patient has respired 100 % O_2 for 3 to 5 minutes.

EXERCISE

A couple of minutes before the end of a usual clearance period an intramuscular injection of PAH was made into the gluteal region. Our usual adult dose is 6—7 ml of a 20 % PAH solution to which is added three times the same volume of isotonic saline and 1 ml of novocain to prevent the rather intense pain otherwise caused by the injection.

After a rest period of 20 minutes the intravenous drip was interrupted and the catheter removed. The subject has been made to walk in a double

laboratory stair with three steps. The hight between the steps has been 14 cm. The pace has been one step per second to the beats of a metronome.

The amount of work done varies with the weight of the subject and can not be accurately calculated. It probably amounts to some few hundred kilogrammeters per minute. This represents a comparatively slight muscular effort. Grande Covian and Rehberg in their study of the effect of exercise on the renal function (creatinine clearance) used from 700 to 1550 kilogammeters per min. The smallest work used by these authors, represents at least twice the amount done by our subjects. This period usually lasted for 20 minutes. Sometimes it had to be interrupted after a shorter period. Blood for PAH, inulin, creatinine, and urea estimations was drawn just before and just after the period and the value found by interpolation. Urine was obtained by a repeated catheterization. Most normal subjects do not react much on this degree of work, although we have seen healthy subjects become quite exhausted.

It must be remembered that the subjects in the Danish publications on the effect of muscular work were well-trained young men. Various circumstances which will not be discussed in this paper have made us try another method where the work is done in the recumbent position as used by Merril and Cargill.

A single intramuscular injection of PAH has proved effective for maintenance of a constant blood level.

RESULTS

So far we have made 50 tests in 43 individuals. PAH, inulin, endogenous "creatinine", and urea clearance have been determined simultaneously. During the first months we sometimes got too high blood levels of PAH, particularly in cases where the renal blood flow was reduced. As to the results shown below the concentration in the blood in all instances was below the level at which a self depression is apt to occur. Table I shows some of the results obtained in *normal subjects*. It will be seen that the values are within the limit of those given by Goldring and Chasis (1944). According to these authors the normal inulin clearance in males and females varies from about 100 to 150 ml per minute corrected to 1.73 sq. m. body surface area. The same corrections have been made for all values in the present publication. Goldring and Chasis found the average inulin clearance for 67 males 130 ml per minute and for 21 females 115 ml per minute. The PAH plasma clearance for both sexes varied from 500 to 830 ml per minute, i. e. an average of 700 ml per minute for 61 males and 600 ml per minute for 17

Table I. *Normals.*

Subject	Born	Plasma clearance ml/min.				Renal blood flow	Filtration fraction
		PAH	Inulin	End. "creat."	Urea		
G.H. ♀	1905	705	98	86	79	1150	13.8
M.M. ♀	1913	685	143	119	116	1140	20.9
D.J. ♀	1911	594	141	113	93	912	23.8
G.J. ♀	1922	646	111	100	52	1050	15.5
J.J. ♂	1903	579	132	118	63	1000	23.9
S.Z. ♂	1899	789	149	113	64	1360	18.9
I T. ♂	1923	910	155	131	82	1540	17.1
Average:		701	133	111	79	1165	19.1

females. Similar values were found by A. J. Merrill for 35 normal individuals and by Mokotoff, Ross, and Leiter for 14 normal males and females. On the basis of these figures it follows that the average renal blood flow is 1200 ml per minute in males and 1050 ml per minute in females, varying from 850 ml to 1450 ml per minute. According to the same authors the maximal rate of tubular excretion of PAH (Tm PAH) varies from 65 to 90 mg per minute.

The renal circulation no doubt reacts readily to apparently slight stimuli. In that connection it may be mentioned that Homer Smith in a few instances found a considerable fall in the renal blood flow as a result of anxiety. It seems that considerably smaller stimuli than those used by Trueta and co-workers in their famous experiments on rabbits may produce significant changes in the renal blood flow. George A. Wolf in 1943, published some studies on the effect of pain on the renal blood flow. Cranial pain was produced by means of a screw arrangement around the head. Intense pains thus induced in four cases caused a fall in the renal blood flow to 27 per cent, 58 per cent, 27 per cent, and 72 per cent, respectively, of the original values (20-minute periods). The renal blood flow was measured by means of diodrast. The inulin clearance fell somewhat less, viz., to 50 per cent, 95 per cent, 55 per cent, and 90 per cent, respectively, of the original values. The consequent rise in the filtration fraction thus indicate an efferent spasm. No effect was observed when one hand was immersed in cold water (5—8° C). This probably represented too weak a pain stimulus.

We have made *one experiment* which in this connection may be worth mentioning: In a 54 years old debile epileptic with hydrocephalus, who presented no evidence of cardiovascular or renal disease, a tourniquet was applied to one of the thighs so that the arterial pulse disappeared. The torniquet was applied after two normal clearance periods and the blood supply then cut off for two subsequent periods of 10 minutes after which the tourniquet was removed and a final normal clearance period carried out. The PAH plasma clearance was 540 ml per minute during the first two periods and on the whole rather low. During the first period with the tourniquet applied the PAH clearance was 550 ml per minute, 485 ml per minute during the subsequent period, and 409 ml per minute during the final normal period. The inulin clearance was normal, and so was the urea clearance. The slightly lowered values for PAH plasma clearance with the lowest one following the removal of the tourniquet can probably just as well be ascribed to anxiety as to the tourniquet. A filtration fraction of 29 (normal 15 to 25) indicates constriction of the efferent vessels. St. E. Bradley has made a similar experiment with pressure around the waist which make one think of the technique at the roentgenological procedure during intravenous urography. We have on the whole made several observations which strong indicate that psychic factors play an important role for the renal blood flow and filtration rate.

The following Table II shows the results in some of our *cardiac subjects at rest.* A striking feature is the often considerable reduction in renal blood flow and a comparatively slightly impaired glomerular filtration

Table II. *Cardiac Patients.*

Subject	Born	Date	Plasma clearance ml/min.				R.B.F.	F.F.	Clinical notes.
			PAH	Inulin	End 'creat.'	Urea			
P.P. ♀..	1878	16/8—48	339	59	64	51	522	17.2	*Aortic stenosis*, arteriosclerotic. Syncope, dyspnea on exertion. Slight edema. X. Ray: Enlarged left ventricle. Arteriosclerotic aorta. Heartvolume: 440 ml/sq.m. No pulmonary congestion. No digitalis or mercurgan medication.
K.J.O. ♂	1891	7/6—48	319	109	84	65	525	34.2	*Aortic stenosis*, rheumatic. Advanced stage with orthopnea and cyanosis. No edema. X. Ray: *Enlarged left ventricle. No pulmonary congestion.* Digitalis medication. Died Sept. 29, 1948: Cardiac hypertrophy and dilatation. The aortic ostium only open for a pencil. Calcareous deposits.
O.P. ♀..	1882	18/8—48	335	104	66	61	613	29.3	*Aortic regurgitation*, rheumatic. Slight dyspnea on exertion. Slight edema. X. Ray: Elongation of aorta. Heart volume: 351 ml/sq.m. Normal configuration. No pulmonary congestion. Auricular fibrillation. Digitalis medication.
R.J. ♂..	1884	18/6—48	455	86	86	56	786	18.4	*Aortic regurgitation*, luetic. Slight dyspnea on exertion. No edema. X. Ray: Enlargement of the left ventricle. No pulmonary congestion. No digitalis medication.
H.R.♀..	1906	22/10—48	495	97	104	63	813	17.3	*Mitral stenosis.* Dyspnea on exertion. No edema. X. Ray: Heart vol. 330 ml/sq.m. No pulmonary congestion. No digitalis medication.
W.A.♂ .	1889	24/6—48	269	97	58	35	449	36	*Mitral stenosis.* Advanced stage with orthopnea, cyanosis, and marked edema. Auricular fibrillation. X. Ray: Cor bovinum, pulmonary congestion. Improvement followed digitalis and mercurgan medication, edema disappeared (Aug. 20th). Further improving (Sept. 15th).
		20/8—48	411	99	81	48	638	24	
		15/9—48	431	125	86		720	29	
V.F.H.♀	1885	9/6—48	47	18	21	11	81	38.4	*Mitral stenosis.* Advanced stage with orthopnea, cyanosis, and marked edema. Auricular fibrillation. X. Ray: Considerable general enlargement of the heart. Pulmonary congestion. Digitalis and mercurgan medication. Dies Aug. 24th, 1948: Hypertrophy and dilatation of the heart; weight 580 g. Marked mitral stenosis. Right kidney: Large calculus, pyelonephritis. Left kidney normal. Blood pressure normal.
P.R.L.♂	1920	9/9—48	538	113	116		925	21	*Coarctation of aorta.* Verified at operation Sept. 29th, 1948. Dyspnea on exertion. No edema. Blood pressure: Lower extremity 135/95, upper extremity 170/95. X. Ray: Slight enlargement of the heart. No pulmonary congestion.
J.O. ♂..	1880	25/10—48	533	94	121	54	920	19.4	*Total A·V block.* Arteriosclerosis. Heart action 28. No edema. X. Ray: Enlargement of the heart, normal configuration. No pulmonary congestion.

rate and consequently a high filtration fraction. On the whole there is a correlation between the clinical condition and the renal function.

These marked reductions in PAH clearance and renal blood flow may naturally arise the question as to the *validity of the method* in these conditions. It seems quite reasonable to expect that the hypoxemia which must be assumed to be present in the renal tissue might involve a change in the tubular function, e. g., a lowered self depression limit or a reduced extraction fraction.

For the same reason one could claim determinations of the self depression limit and PAH Tm necessary. Determinations of PAH Tm occasionally have been carried out, but not to the extent we really desired, owing to a sparce supply of PAH. The validity of the PAH clearance as a measure of the renal blod flow primarily depends on the ability of the kidneys to remove the normal fraction of PAH during one passage through the kidney, i. e., whether the extraction fraction is normal. The normal extraction fraction for PAH at low blood levels (preferably under 3 mg %, at least under 6 mg %) varies from 88 to 100 per cent averaging 92 per cent (St. E. Bradley). This is what makes the method applicable for measuring the renal blood flow. The question than arises whether the extraction fraction is normal also in cases of cardiac failure, particularly the extreme forms. So far we have not carried out such determinations of the extraction fraction. A. J. Merrill, however, has determined the extraction fraction by means of catheterization of the renal vein in patients with cardiac failure and found it entirely normal. In 9

patients he found a average extraction of 88 per cent. The extraction was normal even in patients with severe failure and a renal blood flow as low as 175 ml per minute. Similarly Van Slyke by the same technique found normal extraction fraction in shocked patients with a renal blood flow as low as 3 per cent of normal, i. e., an extremely low renal blood flow. St. E. Bradley in 14 patients with hypertension found normal extraction fractions in 10 cases, and only in 2 cases with uremia he found markedly lowered values. Brannon, Merrill, and Warren found normal extraction in chronic anemia with markedly reduced renal blood flow.

These findings we think can be taken as conclusive a to the validity of the PAH method in cardiac failure. The lowered PAH clearance values consequently indicate a lowered renal blood flow.

We do not, however, intend to draw conclusions as to the renal blood flow in various degrees of cardiac failure from this preliminary report. Nor will any attempt be made to discuss the partial functions in detail. This will, however, be considered in a later publication.

Our intention here is merely to point out the fact that the glomerular filtration rate may be but slightly reduced despite a markedly reduced renal blood flow. This is further illustrated by a high filtration fraction. It must be assumed that the kidney possesses an active mechanism which compensates a reduction in blood flow by contriction of the efferent artery. A more marked reduction of the filtration rate is seen only in marked cardiac failure, but even in these subjects the filtration rate is considerably les impaired than the renal blood

Table III.   *The Effect of Exercise in Normal and Cardiac Subjects.*

Subject	Born	Date		Plasma clearance ml/min.				R.B.F.	F.F.	Clinical notes
				PAH	Inulin	'Creat.'	Urea			
I.T. ♂.	1923	5/10—48	rest	910	155	131	82	1540	17.1	*Normal.* Exercise for 23 minutes: Unaffected.
			exercise	591	145	97	72	1000	24.5	
G.H. ♀	1921	20/10—48	rest	812	184	163	58	1220	22.6	*Normal.* (Hb. 78 %) Exercise for 20 minutes: Exhausted, dyspnoic, sweating.
			exercise	143	47	38	13	214	32 8	
K.K. ♀	1925	19/10—48	rest	772	173	128	70	1265	22.3	*Normal.* Exercise for 20 minutes: Very exhausted, dyspnoic.
			exercise	326	89	76	26	535	30.0	
H.L. ♂	1920	7/10—48	rest	600	134	98	61	1100	22.3	*Calcareous deposits, bandformed, in the pericardium.* Slight dyspnea on exertion. No edema. X. Ray: also hypertrophy of the left ventricle. No pulmonary congestion. *Exercise* for 18 minutes: Highly dyspnoic, sweating.
			exercise	525	134	92	49	922	25.4	
H.N. ♀	1884	29/9—48	rest	495	85	65	46	812	16.7	*Aortic regurgitation,* luetic. Dyspnea on exertion. No edema. X. Ray: Moderate enlargement of the heart. 443 ml/sq.m. *Exercise* for 18 minutes: Very exhausted, had to rest for ¾ — 1 minute several times. Dyspnoic, sweating.
			exercise	270	71	53	50	443	26.4	

flow. Inulin clearance, endogenous "creatinine" clearance, and urea clearance apparently follow each other closely.

Table III and IV show the results in some *normal and cardiac subjects during muscular exercise and hypoxemia.* Our material does not yet allow definite conclusions, but we hope to be able to present more extensive and conclusive data in a later publication. There are, however, so far two outstanding features. Firstly, there is the fact that the PAH clearance and the renal blood flow not in a single instance with hypoxemia have showed a fall, but always an increase, in some instances even a marked one.[1] Secondly, a considerable fall in PAH clearance and renal blood flow has been observed during muscular work, not in cardiac subjects only, but even in normal individuals. The filtration fraction increases when the blood flow decreases. It falls when the renal blood flow increases during hypoxemia.

The clearance values for inulin, creatinine, and urea do not follow the PAH celarance during hypoxemia, but remain normal. During exercise they may fall, but not to the same extent as the renal blood flow.

Our present investigations demonstrate that the kidney may show a highly different reaction to various conditions in which an increased cardiac output is a common feature.

We will not at the present discuss the various mechanisms which may be responsible for this reaction. We have, however,

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[1] Later on we have found during hypoxemia a considerable fall in the renal blood flow in aortic stenosis.

Table IV. *The Effect of Hypoxemia in Normal and Cardiac Subjects.*

Subject	Born	Date	Period	Min.	Plasma clearance ml/min				R.B.F.	F.F.	Clinical notes.
					PAH	Inulin	'Creat.'	Urea			
C. J. ♀	1922	27/10 — 48	rest	20	646	111	100	52	1050	15.5	*Normal* (Hb 82 %).
			9.5 % O₂	20	700	97	100	52	1135	13.7	Cyanotic, otherwise unaffected.
K. K. ♀	1925	15/10 — 48	rest	20 5	565	151	149	91	928	26.7	*Normal.* Cyanotic, otherwise
			9.5 % O₂	20	800	133	(198)	109	1310	16.6	unaffected.
H. R. ♀	1906	27/10 — 48	rest	20	495	97	104	63	813	17.3	*Mitral stenosis.* Dyspnea on exertion. No edema. X. Ray: Heart vol. 330 ml/sq. m. No pulmonary congestion. No digitalis medication. Great distress and cyanosis developed during the hypox. test, which had to be interrupted after 12 minutes.
			9.5 % O₂	12	525	100	104	41	860	19.0	
			rest	12	465	88	94		764	18.9	
H. S. ♀	1895	24/11 — 48	rest	15.5	513	79	97	75	827	17.0	*Aortic regurgitation + stenosis,* rheumatic. Dyspnea on exertion. Slight edema. X. Ray: Heart vol. 308 ml/sq. m., normal configuration. No pulmonary congestion. Digitalis medication prior to admission. Great distress and dyspnea developed during the 3rd period so that the test had to be interrupted after 6¾ minutes.
			9 5 % O₂	10	532	79	97		860	14.9	
			9.5 % O₂	6 ¾	580	76	95	74	935	14.4	
			rest	7	518	85	92		836	16.4	

started another series of experiments which may contribute to the understanding of this problem.

Table V and VI show the results in some patients with *chronic anemia* and in patients with *thyreotoxicosis.* The tests have been repeated after the return to normal of hemoglobin values and basal metabolism in the respective groups.

As previously mentioned, PAH clearance and renal blood flow have been studied by Bradley and Bradley in patients with chronic anemia, but so far no reports dealing with the relation between basal metabolism and renal blood flow have appeared. Sufficient data have so far not been obtained though

our results also here may serve to demonstrate some outstanding features. Firstly we find a reduced renal blood flow during anemia with return to normal as soon as the hemoglobin percentage is sufficiently elevated. Secondly we find increased renal blood flow with increased basal metabolism. In one patient the renal blood flow falls to normal when the basal metabolism falls. In another patient there also is a fall in renal blood flow, but not so striking. On this patient a subtotal strumectomi had been performed, whereas the first patient was treated with methylthiouracil. The second case will be further studied.

Table V. *Anemia.*

Subject	Born	Date	Plasma clearance cc/min.				R.B.F.	F.F.	Hb%	R.B.C.	H.crit.	Diagnosis.
			PAH	Inulin	'Creat.'	Urea						
G.B. ♀	1926	13/9 −48	863	156	106		1090	18.1	53		20.5	*Secondary anemia.* Gastric ulcer, melena.
		23/11−48	897	128	131	70	1575	14.3	108		43	
G.M. ♀	1876	18/8 −48	511	89	63	65	609	17.5	42	1.62	16	*Pernicious anemia* + debilitas senilis, excited and paranoic.
		16/11−48	439	84	84	56	732	19.1	93	5.01	40	
K.Aa. ♂	1879	23/6 −48	446	119	69	58	566	26.7	53		21	*Secondary anemia.* Cancer of the stomach.

Table VI. *Thyreotoxicosis.*

Subject	Born	Date	Plasma clearance cc/min.				R.B.F.	F.F.	Basal metabolism	Clinical notes.
			PAH	Inulin	'Creat.'	Urea				
T.A. ♀	1915	11/6 −48	903	163	108	91	1385	18	150 %	Methyl thiouracil since June 15th, 1948.
		17/9 − 48	697	161	116	135	1070	20 5	100 %	
E.T. ♀	1920	8/10−48	927	121	81	95	1570	13.1	154 %	Oct. 28th, 1948: Strumectomia subtotalis.
		12/11−48	948	142	113	81	1605	15.0	86 %	

On the whole this is another example of a different response of the kidney to circulatory changes with an increased output as a common feature.

A *theoretical explanation* may easily be found to several of the changes in renal blood flow reported in this paper. The lowered renal blood flow during exercise may be explained as a result of an increased demand for blood by the active muscles with their dilated capillaries. In chronic anemia an active reversible constriction of the renal vessels and a consequent shunting of the blood to more vital organs may be imagined.

All these changes, however, must depend on a special mechanism confined to the kidney. It is quite obvious *why* these functional changes take place, but we are far from knowing *how*. Attempts to solve this problem are going on in our hospital.

SUMMARY

This is a preliminary report on experimental investigations of renal function and renal hemodynamics in normal and certain pathological conditions.

The normal values of inulin clearance, endogenous "creatinine" clearance, urea clearance and the clearance of sodiumparahippurate were found within the same limits as reported by previous writers.

In heart failure the renal blood flow frequently was found pronouncedly reduced. The glomerular filtration rate was usually

reduced to a lesser degree. A correlation between clinical condition and renal function and hemodynamics was commonly present.

During experimental hypoxemia (respiration in 9.5 % O_2) normals and cardiac patients frequently showed an increase in renal blood flow. Cases with stenosis of the aortic valve may react differently.

During muscular exercise renal blood flow in normals and cardiac cases usually declined. The filtration fraction varied ·during hypoxemia and muscular work in-

versely of renal blod flow. In anemic conditions a reduced renal blood flow was demonstrated, and in hyperthyroidsim an increased RBF. In both conditions a return to normal values concomitant with clinical improvement was observed.

It is stressed that different conditions with increased cardiac output as a common feature may show divergent renal hemodynamics.

The problems connected with these experimental results will be discussed in coming papers.

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SERUM PROTEINS AND AGGLUTININS IN A CASE
OF TYPHOID FEVER WITH SEVERE HYPOPROTEINEMIA

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From a functional point of view the proteins in the organism may be divided into two different classes. The first group comprises those which are characteristic constituents of the cells and are essential for the specific cellular functions. The second consists of the proteins in blood plasma and extracellular fluid. They have several functions, for example their rôle in maintaining the fluid equilibrium of the body and their faculty of acting as a depot of proteins which supplies material for the normal protein metabolism. There is, moreover, an intracellular reserve, mainly located in the liver, of non-specialized proteins and polypeptides which may be required by an increased demand for material to synthesize the specific proteins.

When the nitrogen equilibrium is negative the organism as long as possible endeavours to maintain the supplies of the specific organic protein and the plasma protein at an adequate level; instead the reserves are gradually depleted. Only when these are completely emptied it is possible to register a reduction in the other protein groups. The clinical sign of such a condition is hypoproteinemia which is a serious symptom as it is manifested only in the advanced stages of an impaired nitrogen metabolism.

Hypoproteinemia might arise for several very different reasons. It may be due to insufficient intake, e. g. in cases of starvation or when the diet is poor in proteins. Inadequate resorption in digestive disorders can have the same result. Febrile conditions and other wasting diseases may cause an increased break down of proteins resulting in hypoproteinemia. In cases of extensive burns the protein losses may become so large that they cannot be replaced by the synthesis. Apparently hormonal factors also can influence the protein equilibrium. Thus Lewis and McCullagh (1947) have demonstrated that the serum albumin and the γ-globulin are reduced in Cushing's disease.

However, one of the most common causes of hypoproteinemia is an inadequate supply of food. In modern times as well as in bygone days disasters, in the form of elemental catastrophes and war, that have befallen mankind, have afforded frequent opportunities of studying the consequences of such nutritive inadequacies. A current report on this subject is given by Hottinger and his colleagues (1948).

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When nutritional conditions and in particular the supply of proteins return to normal the serum protein values are gradually normalized. We know now that not only the quantity but also the quality of the proteins supplied with the food are of decisive significance in a complete recovery. Thus, the composition of the amino acids in the supplied proteins are of the utmost importance. Most of our knowledge of these matters is based on experimental studies in animals carried out especially by Whipple and his associates (1948).

Studies by Heidelberger & Pedersen (1937) and Tiselius & Kabat (1939) have informed us that antibodies are modified globulins and therefore we could easily surmise that there is some connection between the formation of antibodies and nutrition. Especially Cannon and his colleagues (1948) have studied this problem in detail. They have investigated the formation of antibodies in animals under various circumstances finding that there is a close relationship between the antibody response to an antigen and the nitrogen equilibrium of the body. Wissler, Woolridge, Steffee and Cannon (1946) have demonstrated that the organism requires an adequate supply of essential amino acids with the food in order to manufacture antibodies in sufficient quantities. Cannon (1945) points out that immunoglobulin contains at least seven of the amino acids which, being essential, cannot be synthesized by the body but must be supplied with the food. Schoenheimer, Ratner, Rittenberg and Heidelberger (1942) have studied these problems using labelled amino acids and have shown that if such acids are administered perorally a large percentage of them

quickly enters the immunoglobulin molecules. A matter deserving due consideration in this connection has been pointed out by Miller (1948). If rats are starved the result will be reduced activity of the liver enzymes and he attributes this reduction to a decrease in the enzyme protein *per se* and not to a loss of prosthetic groups or activators.

The results of these and many other investigators have conclusively proved that the proteins and amino acids supplied with the food play a most important rôle in the metabolism of proteins in the most comprehensive sense of the term and consequently also in the synthesis of immunoglobulin.

However, the danger should be avoided of drawing too many conclusions from experimental results won by studes of serum proteins and antibodies. The humoral immunity factors demonstrable by current methods constitute only one fraction of the combination of defensive forces that confer on the organism its real resistence to infection and other external influences. Much indicates that intracellular factors might be just as important.

It may be taken for granted that the course of infectious diseases is to a high degree dependent on the available immuno-chemical resources, which cannot in their turn be maintained without an adequate diet, especially with respect to proteins. Many authors have emphasized the importance of reinforcing the protein metabolism in these diseases by alimentary measures (Cuthbertson, 1948). A striking, often quoted example of this is the greatly improved result of the treatment of typhoid fever won by abandoning the previously enforced starvation régime in favour of a comprehensive

diet rich in calories and proteins (Coleman, 1917).

In view of the interplay described above between the metabolism of proteins and antibody synthesis the following case history is highly illustrating.

On June 30th, 1947, a 29 year old man was infected with typhoid fever on a boat trip. About 60 other passengers became ill at the same time. The patient had earlier been repeatedly inoculated against typhoid fever, the most recent occasion in 1943 (the patient's own information). On July 20th he came down with chills and a high temperature and was admitted to an epidemic hospital on July 21st. There the examination revealed that the spleen was abnormally enlarged and that the patient despite the high temperature (40° C.) only had a pulse of 82. A blood culture on July 23rd exhibited a plentiful growth of *Eberthella typhosa*. On the same occasion Widal's reaction showed a typhoid-H-agglutination in a dilution of 1 : 50, but it was negative in other respects. The patient then went through a severe but on the whole typical typhoid fever. Towards the end of August the temperature had fallen to subfebrile values and the acute phase of the disease was apparently over.

During the convalescence the patient, after his initial improvement, again became worse, lost appetite and frequently vomited and had diarrhoea. He complained of difficulties in swallowing and an X-ray examination revealed an ulcerative process in the esophagus. Cultures from the faeces gave negative results in respect of typhoid bacilli. Nothing indicated a relapse of the typhoid disease. As time went by the patient's attitude to food became more and more negative and in consequence of this and the digestive disorders the patient gradually developed hypoproteinemia with a serum protein level of about 3 per cent. Large nutritional edemas developed and in this condition the patient was admitted to the Medical Clinic of the University Hospital, Uppsala, on November 5th, 1947 (hospital record: Med. Clin. 11/48).

There the patient was given blood and plasma transfusions at short intervals. Dextran, a plasma substitute, and protein hydrolysates were also ad-ministered but there was no sign of improvement; the patient on the contrary became worse with increasing edema and periodic attacks of hunger tetany which promptly subsided following calcium injections.

Towards the end of January, however, the scales turned, whether *post* or *propter* the therapy is difficult to determine. The desire for food returned and the patient ate everything given him with an enormous appetite. He was given a high caloric diet, rich in proteins. Little by little the serum protein increased, the edemas lessened and were excreted via the kidneys. In connection with the disappearance of the edema the patient's weight decreased about 30 kg (from 89 to 58 kg). After about a month the serum protein level was more or less normal and the patient improved steadily. The weight curve again exhibited a relatively rapid rise, this time for normal reasons and not because of edema. At the end of March the patient fell ill with hepatitis. Owing to the fact that the patient approximately 100 days earlier had received a large number of plasma transfusions, it very probably was a case of homologous serum hepatitis. The disorder had a very benign course, however, and on April 29th the patient was well enough to be sent home. Later on the patient told us in a letter that he after about a month felt well again and was able to carry out his usual work.

On November 6th, 1947, during the hypoproteinemic phase and a little more than three months after the beginning of the disease, Widal's reaction was made as a matter of routine. It was negative (i. e. the titre was lower than 1 : 50). During the continued course of the diesase the agglutination reactions were recorded, and in connection with the increase in total protein there was a marked increase of the typhoid agglutinin titre. On four occasions electrophoretical and ultracentrifugal examinations of the patient's serum were carried out as a part of a programme for studying serum proteins instituted by Professor Jan Waldenström and Doctor Kai O. Pedersen, Uppsala, to whom the author wishes to express his gratitude for their kindness in making available the protocols.

For the sake of lucidity the results of the various examinations have been set upp in tabular form.
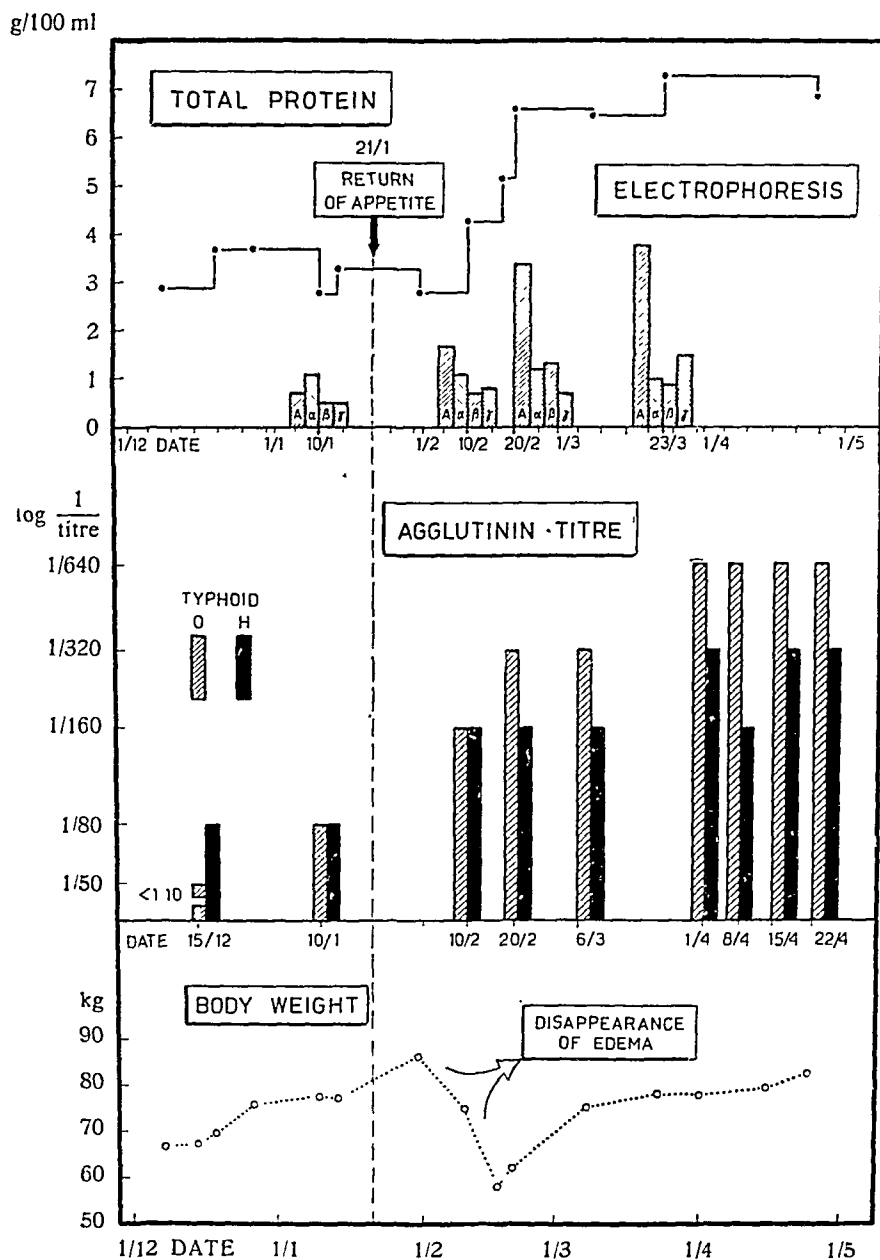
Fig. 1.

Table I.

| Date | Serum protein fractions in Gm. per 100 ml. | | | | | | | | | | |
| | Sodium sulphate fraction-ation and Kjeldahl analysis | | | Electrophoretical analysis | | | | | Ultracentrifugal analysis | | |
	Total protein	Alb.	Glob.	Alb.	α	β	γ	α+β+γ	4.5 s.	7 s.	20 s.
10/1	2.8	1.1	1.7	0.7	1.1	0.5	0.5	2.1			
10/2	4.3	2 2	2.1	1.7	1.1	0.7	0.8	2.6	2.8	1.4	
20/2	6.6	4.1	2.5	3.4	1.2	1.3	0.7	3.2	5.1	1.2	0,0
23/3	7.3	4.8	2.5	3.8	1.0	0.9	1.5	3.4	5.1	1.8	0.4

Table II.

| Date | Serum protein in Gm. per 100 ml. Kjeldahl analysis | Agglutination titre | | | | |
| | | Typhoid | | | Paratyphoid B | |
		O	H	Vi	O	H
9/10	2.8					
20/10	2.8					
5/11	3.3					
6/11		1 : 50	1 : 50		1 : 50	1 : 50
20/11		1 : 10	1 : 80			
22/11	3.0					
30/11		1 : 10	1 : 80			
8/12	2.9					
15/12		1 : 10	1 : 80			
19/12	3.7					
27/12	3.7					
10/1	2.8	1 : 80	1 : 80	1 : 20	1 : 40	1 : 10
14/1	3.3					
31/1	2.8					
10/2	4.3	1 : 160	1 : 160	1 : 20	1 : 80	1 : 10
17/2	5.2					
20/2	6.6	1 : 320	1 : 160	1 : 10	1 : 160	1 : 10
6/3		1 : 320	1 : 160	1 : 10	1 : 160	1 : 10
8/3	6.5					
23/3	7.3					
1/4		1 : 640	1 · 320	1 : 10	1 : 160	1 : 20
8/4		1 : 640	1 : 160	1 : 10	1 : 160	1 : 40
15/4		1 : 640	1 : 320	1 : 10	1 : 160	1 : 20
22/4		1 : 640	1 : 320	1 : 10	1 : 80	1 : 20
24/4	6 9					
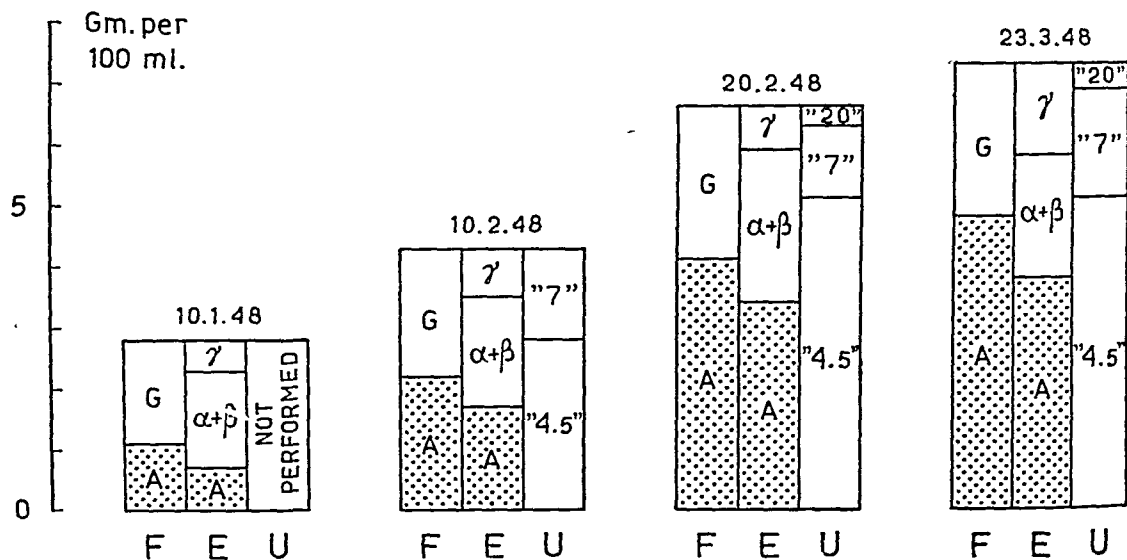
Fig. 2. The relations between the different serum protein components schematically arranged according to Waldenström. F = 22.5 per cent sodium sulphate fractionation and Kjeldahl analysis. E = electrophoretical analysis according to Tiselius. U = ultracentrifuge analysis according to Svedberg-Pedersen. A = albumin; G = globulin; "4.5", "7", "20" = s_{20} for the different ultracentrifuge components.

DISCUSSION

We are dealing with a case of typhoid fever which during convalescence developed hypoproteinemia of fairly uncertain genesis. Pickert (1948) has carried out an investigation into the behaviour of plasma protein in typhoid fever finding that it decreases slightly during the first phase of the disease to return to the normal values after the end of the febrile period in most cases. Consequently the decrease would reach its lowest level during the 3rd or 4th week of the disease. In the case of our patient the hypoproteinemia lasted far longer and would therefore seem to be not characteristic of typhoid conditions. It was thought to be of a secondary character and due to inadequate nutrition and impaired resorption.

The electrophoretical examinations show how the different protein fractions reacted at different times. When the total protein concentration in the serum was at its lowest this was mostly due to the severe albumin reduction, whereas the total globulin value was very nearly normal. Subsequenty the albumin returned to normal levels. The globulin also increased and reached a value rather above normal. During the hypoproteinemic stage there consequently was a condition of relative hyperglobulinemia which gradually lessened as the conditions otherwise improved. This course of the regeneration process fits in well with Rossiter's (1946) observations on starving prisoners of war, in whome he studied the serum protein when they were given a rich comprehensive

diet. Zeldis, Alling, McCoord and Kulka (1945) have made similar observations in dogs subjected to starvation. They also found that the α-globulin as a rule was increased during the period of starvation.

If we consider the various globulin fractions in our case we find similar conditions. All the time the α-fraction was somewhat increased. The β-fraction exhibited a slightly more rapid increase during the period when the total protein went up most markedly. When stabilization set in it again fell slightly. The γ-globulin rose to a top value way above normal.

Enders (1944) has demonstrated that the typhoid-H-agglutinins belong to the γ-fraction while he finds the O-agglutinins in a fraction that mainly consists of β-globulin. On considering the electrophoresis values from our patient in this light we find no provable connection between the β-fraction and the O-agglutinin values while the curves representing the increase in the γ-fraction and the H-agglutinin titre correspond fairly well.

What might then be the reason for this late and unexpected increase in the agglutinin titres? Since no relapse of the disease could be observed, but instead a very marked improvement it appears very improbable that it was a case of so called "secondary response", i. e. the rapid and considerable titre increase obtained when an alredy immunized organism re-contacts the same specific antigen (Burnet, 1941).

One explanation might be that the increased titre implies a so called "anamnestic reaction". This means that a specifically immunized organism can produce antibodies through other incitements than the specific antigen. Such a reaction is sometimes caused by the influence of a totally different antigen or even by non-antigenic substances. As we remember our patient later came down with a hepatitis of the homologous serum type. This form of hepatitis has a period of incubation of approximately 100 days. The unexpected increase of the aglutinins would therefore seem to have taken place when the patient was in the incubational stage of the liver disease. Consequently the patient was under the influence of a new antigen during this period, and there we have the conditions required for the generation of an "anamnestic reaction". This type of non-specific antibody response does, however, as a rule arise only when the actual immunization against the new antigen already has started, i. e. when the disease has become manifest. During the period of incubation the stimulation of the antigen on the formation of antibodies is probably not strong enough to start this process. In the case of typhoid, to take an appropriate example, the agglutinins cannot be demonstrated until the second week of illness. Consequently, in our case the specifically stimulating effect caused by hepatitis virus probably was very weak or even absent during this period. It therefore seems unlikely that this virus could have been the cause of a lively synthesis of apparently quite aspecific antibodies.

Another form of "anamnestic reaction" sometime turns up some time after large hemorrhages. In such cases the protein synthesizing cells are stimulated to a lively activity (Basu & Sudhindra, 1947) and this stimulation would seem to cause the immunoglobulin producing centres to reproduce

specific globulins of a type which they have produced on an earlier occasion. The hypoproteinemia following the hemorrhage might therefore be a factor setting off this type of "anamnestic reaction".

The present case can perhaps be similarly interpreted. As we know the patient was in a state of severe protein deficiency and the synthesis of serum protein and also of immunoglobulin was much reduced. When the nutritional state of the patient became normal ·the synthesis of proteins increased, and the cells or cell structures which under the influence of the typhoid antigens had been stimulated to a production of typhoid agglutinins in that connection again increased their output of the specific globulins. This would therefore be interpreted as a case of "anamnestic reaction" caused by alimentary stimulation following a condition of protein deficiency.

SUMMARY

After a brief review of the immunobiological importance of the nutrition, particularly of the proteins in the diet, a report is given of a case of hypoproteinemia caused by dysnutrition and digestive disorders during the convalescence following typhoid fever. Since on recovery the serum proteins again return to normal a simultaneous increase is obtained of typhoid and paratyphoid agglutinins. The reasons underlying this "anamnestic reaction" are discussed.

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ACQUIRED HEMOLYTIC JAUNDICE

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Since the excellent descriptions of an acquired type of hemolytic jaundice given by Widal, Abrami and Brûlè (1907 a, b, c, 1909, 1912), widely divergent opinions and theories on the nature of this disease and its relation to the familial, congenital type described by Minkowski (1900) and Chauffard (1907) have arisen.

Some investigators have doubted the existence of this type of hemolytic jaundice (Gänsslen, 1927), especially when cases assumed to be acquired were revealed by thorough examination of the famliy to belong to the congenital type. (Campbell and Warner, 1925).

Nägeli (1919) held the opinion that both types were due to a primary defect of the erythron resulting in the formation of spherocytes, the difference between the two types being quantitative in this respect. Dawson of Penn (1931), Vaughan (1936) and Tileston (1922) among others expressed a similar view.

Dameshek and Schwartz (1938, 1940) discriminated sharply between familial and acquired types. They produced in guinea-pigs the hematological pictures of acholuric jaundice by the injection of of antiguinea-pig rabbit serum, and since spherocytosis was present in both types, they advanced the theory that hemolytic anemias of all types might be due to the action of "hemolysin".

Doan, Curtis & Wiseman (1935), Gripwall (1938) and Fåhraeus (1939) on the other hand advanced the theory that hemolytic anemia was primarily a splenic disorder, with undue stasis and destruction of blood in the splenic pulp. Minkowski and Chauffard both held the idea of an abnormal function of the spleen.

The existence of two different types of hemolytic jaundice has been confirmed by Boorman, Dodd and Loutit (1946) who demonstrated a well marked serological difference. Using the test of Coombs, Mourant and Race (1945) with an anti-human-globulin rabbit serum for detecting sensitized human red cells, they demonstrated that red cells from patients with acquired hemolytic jaundice were sensitized and could be agglutinated by the reagent whilst the red cells of cases of congenital hemolytic jaundice were not sensitized and could not be agglutinated.

Transfusion experiments have further revealed a fundamental difference. Normal

red cells were found to survive a normal length of time in the circulation of patients suffering from congenital hemolytic jaundice (Dacie and Mollison, 1943), but a very short time in cases of acquired hemolytic jaundice (Loutit, 1946, Loutit and Mollison, 1946). On the other hand the survival of red cells from patients with congenital hemolytic jaundice, in the circulation of normal persons is very short, whilst the red cells from acquired hemolytic jaundice are found to survive a normal time (Loutit, 1946, Loutit and Mollison, 1946).

In the following paper further experiments confirming the existence of acquired hemolytic jaundice as a distinct disease differing fundamentally from the congenital type are presented. These indicate that the destruction of red cells is caused by a normal reticulo- endothelial system removing sensitized red cells. The survival of sensitized red cells transfused to a normal individual showed certain peculiarities, not previously described, which are of importance for the understanding of the hemolytic mechanism.

Case report. A 74 year old male was admitted to Aker Hospital on 10th Febr. 1947. He gave a history of increasing weakness, dizziness, pallor and jaundice of four weeks' duration.

Blood examination revealed a severe anemia with hemoglobin 3.5 g/100 ml and red cells 1 100 000 per cu.mm. Leucocytes 7500 per cu.mm with a normal differential count. Platelets 310 000 per cu.mm and reticulocytes 7.5 per cent. The peripheral blood showed spherocytosis. The extent of microcytosis (spherocytosis) is illustrated by the frequency curve in Fig. 1. The sample was taken after some transfusions had been given. The curve shows that the population of red cells is not normally distributed. By Gerhard Larsen's (1948) method of analysis it is found to consist of 43 per cent normal cells and 57 per cent microcytes. The fragility of the red cells in hypotonic

saline was increased: 0.56—0.34. Examination of the bone marrow showed great increase and hyperactivity of the erythropoietic tissue. The icteric index was 40. The spleen was enlarged to 4 finger breadths below the costal margin. The urine contained increased urobilinogen but no bilirubin. Wasserman reaction negative. Sedimentation rate: 105 mm.

By the use of the technique described by Knisely and Bloch (1945), (binocular, bi-objective microscope focussed on the obliquely illuminated bulbar conjunctiva) the phenomenon of "sludged blood" was demonstrated in the conjunctival vessels.

There was no history of similar disease in the family.
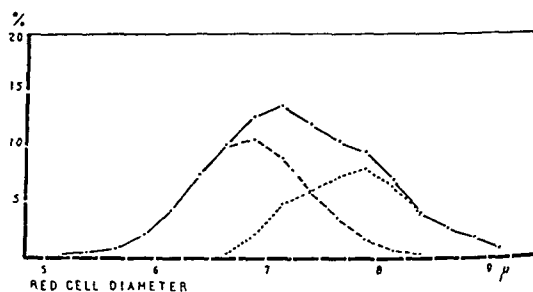
Fig. 1. Frequency curve for the red cell diameter in peripheral blood on Febr. 20th. Analysis by the method of Gerhard Larsen (1948): Mean diameter: 7.25. Standard deviation: 0.72. Main Component: Mean diameter 6.81. Standard deviation 0.51. Frequencies: 57 %. Secondary Component: Mean diameter 7.88. Standard deviation 0.53. Number of frequencies: 43 %. X^2—analysis: 2.34 i. e. P = 0.50. Range of normal mean diameters: 7.80 (7.20—8.35). *Conclusion:* Sample consist of 43 % normal blood cells and 57 % microcytes. Macrocytes not present.

The clinical picture has all the criteria for a diagnosis of hemolytic jaundice; jaundice without bilirubin in the urine, anemia, splenomegaly, spherocytosis, increased fragility of the red cells and reticulocytosis.

METHODS

The test for sensitzed red cells with anti-human-globulin rabbit serum was performed as described by Coombs et al. (1945).

The survival of the red cells in transfusion experiments was followed by the use of Ashby's (1919) differential agglutination technique, using the modifications and improvements suggested by Mollison and Young (1941) and Dacie and Mollison (1943).

SEROLOGICAL EXAMINATION

Agglutination tests ultimately showed the red cells to belong to group A_1MN Rh +. These tests were made difficult by some clumping of the patient's red cells when exposed to a large number of A-sera including the patient's own serum. This reaction was unaltered after adsorption of the sera with the patient's red cells.

"Reverse typing" showed that serum from the patient agglutinated normal red cells of group B, but not of the groups A and O, confirming that his blood group was A.

Three times washed red cells from the patient were strongly agglutinated by anti-human-globulin rabbit serum.

Cold hemagglutinins were not present, and hemolysins could not be detected by the customary tests.

The serum was incubated with normal red cells of group O. The cells were subsequently washed and tested with anti-human-globulin rabbit serum. No agglutination occured.

This lack of demonstrable antibody in the serum in spite of antibody steadily being formed as shown by the Coomb's test and the following transfusion experiments, may be explained by the fact demonstrated by Hill & Haberman that only about 85 per cent of O-cells give a positive result by this test. The serum used for the test was not removed immediately after the blood was drawn, and it is therfore also a possibility that all available antibody in the serum was adsorbed to the red cells before the serum was removed.

TRANSFUSION EXPERIMENTS

The patient needed frequent transfusions for the maintenance of reasonable blood values (refer to Fig. 2). In the course of two months he received the red cells from 26 litres of blood obtained from 53 donors. The plasma was always removed by centrifuging, and the red cells suspended in a small volume of normal saline. Transfusion of the plasma would be dangerous owing to the great volumes of blood needed, and since the donors were of group O the transfusion of incompatible agglutinin was thus avoided.

In the first three transfusions red cells of the same blood group as that of the patient were used, but from the fourth transfusion onwards only red cells of group O N were used. After the fourth transfusion, corresponding to 1 litre of blood, the survival of the transfused red cells in the circulation of the patient was followed (Fig. 3).

Prior to some of the later transfusions, blood was obtained from the patient (indicated by the white part of the columns in Fig. 2) and the red cells subsequently transfused to "normal" subjects (i. e. normal from the point of view of hemolytic anemia), and the survival of the red cells followed. Fig. 4 gives the result of transfusion of red cells from 1.5 litres of blood (obtained 22.—24. March, refer to Fig. 2) to a patient suffering from gastric cancer and anemia caused by hemorrhage. (The relatively large amount of red cells transfused to a
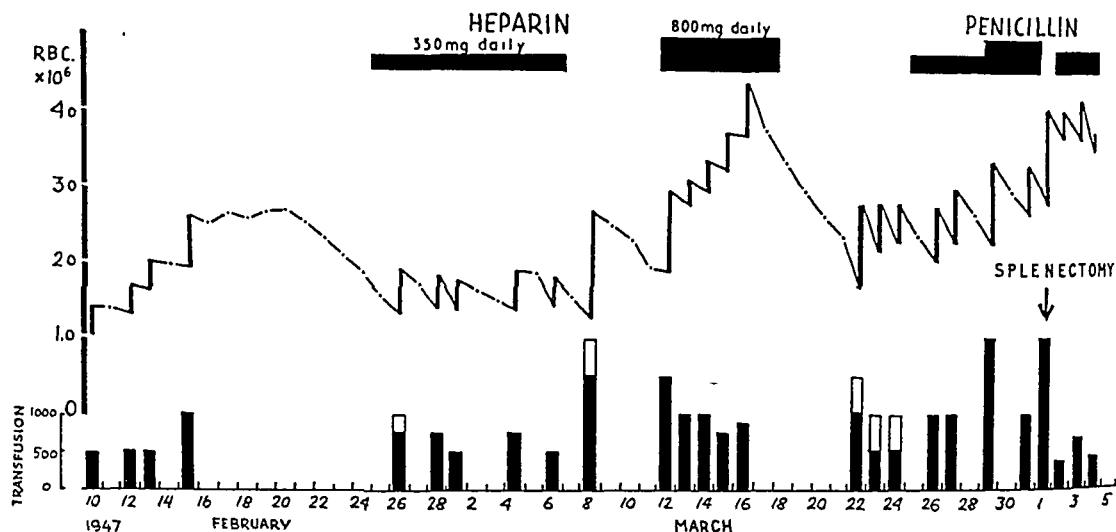
Fig. 2. Male 74 years. Acquired hemolytic jaundice. Red cell count during transfusion treatment White parts of columns indicate withdrawing of blood from the patient prior to the transfusion for the use in transfusion experiments to normal recipients.

RESULTS

patient with low red cell count increases the accuracy of the method). The normal recipient belonged to the blood group A_1N. The fate of the transfused sensitized red cells was revealed by Coomb's developing test. (Refer to Fig. 5). The original red cells from the patient with hemolytic jaundice was traced by anti-M serum. Red cells from the normal donors of blood group ON transfused to this patient were disclosed as *remaining unagglutinated after mixing with anti-M and anti-A serum.* The following scheme illustrates the procedure:

	Blood group
Normal donor	ON
	↓
Patient	A_1MN
	↓
Normal recipient	A_1N

Calculating from the total amount of red cells ·transfused to the patient the rate of destruction was about half a litre of blood per day. The survival experiment illustrated in Fig. 3 corresponds fairly well with this calculation, showing a decrease of the transfused red cells to 50 per cent in 6—7 days. After removing the patient's own group A cells by agglutination with anti-A serum, the remaining unagglutinated group O donor cells were shown, by means of the anti-human-globulin serum, to be sensitized; this sensitization increased during the first three days.

After transfusion of red cells from the patient to a "normal" subject, the sensitized cells disappeared from the circulation of this subject in three days, judged by the anti-human-globulin test. Simultaneously a
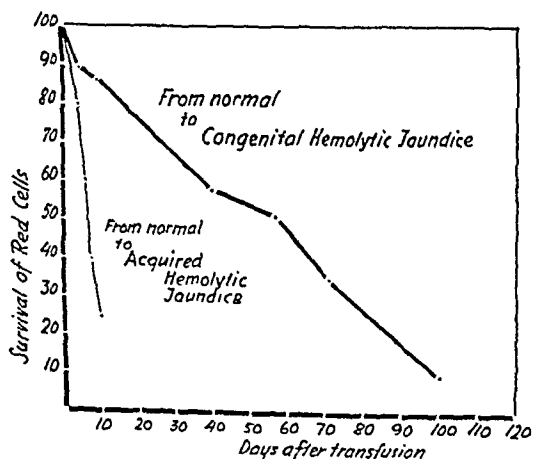
Fig. 3. The survival of normal erythrocytes transfused to the patient with acquired hemolytic jaundice and to a case of congenital hemolytic jaundice.

Fig. 5. The disappearance of the sensitized red cells from the patient with acquired hemolytic jaundice in the circulation of a normal recipient.

Fig. 4. The survival of erythrocytes from the case of acquired hemolytic jaundice transfused to a normal recipient, and from a case of congenital hemolytic jaundice transfused to a normal subject.
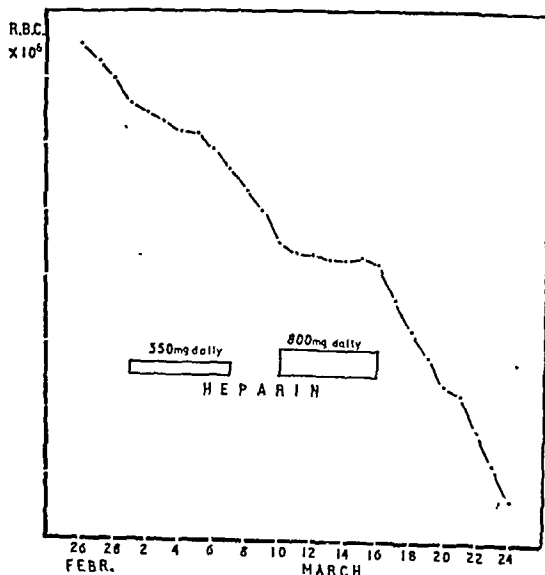
Fig. 6. The influence of heparin on the rate of destruction of red cells in the patient.

rapid fall in the total red cell count occurred (Fig. 5) due to a reduction of the transfused red cells to about 35 per cent of the initial value (Fig. 4). The remainder of the transfused red cells, being unsensitized as checked by the anti-human-globulin test, survived in normal time, about 100—120 days (Fig. 4). (A similar transfusion to a patient with chronic nephritis showed corresponding results.)

The patient was treated with heparin for a short time. Fig. 6 gives the rate of destruction of red cells in the patient's circulation as it appears from the daily red cell count, correcting for the increases by transfusions. It appears that 350 mg of heparin daily was without significant influence on the rate of destruction, whilst an increase to 800 mg daily seemed to cause a diminution or standstill of the destruction although the experiment is not wholly convincing. The heparin treatment had to be stopped after a few days because of an increased bleeding tendency. The withholding of heparin was followed by a severe increase in the rate of destruction.

Treatment with penicillin was without influence on the hemolysis. The patient died of pneumonia a few days after splenectomy, and no significant change in the rate of destruction was observed during this short period.

Figs. 3 and 4 illustrate transfusion experiments in a case of congenital hemolytic jaundice for comparison. He was a man, aged 38, with compensated anemia. He showed the typical features of congenital hemolytic jaundice and familial incidence of the disease. The experiment showed that normal red cells survived a normal time (about 120 days) in the patient's circulation, whilst the patient's red cells survived a maximal time of about 15 days only in the circulation of a normal subject.

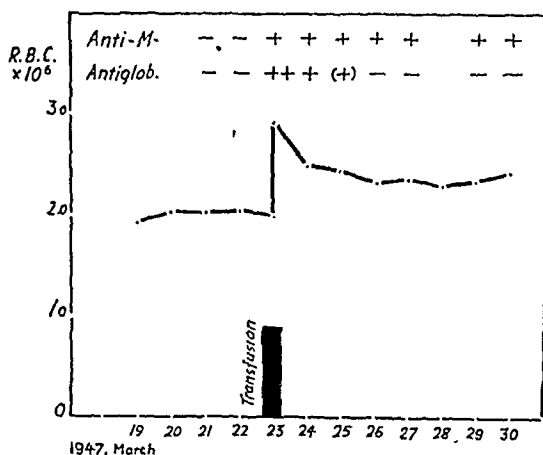The red cells of this patient were not agglutinated by the anti-human-globulin serum.

DISCUSSION

The experiments reported confirm the serological difference between the congenital and acquired type of hemolytic jaundice described by Boorman et al., and corroborate the opinion that this type of acquired hemolytic anemia is caused by a process of immunisation, the immune body being adsorbed on the surface of the red cells making them liable to destruction. In vivo expriments showed the existence of such an antibody, but as in previously reported cases, in vitro experiments failed to demonstrate the antibody in the serum. Possible explanations are either that the time elapsed between collection of blood and separation of the serum has been sufficient for adsorption of all the available antibody by the red cells, or that the O-cells used were of the type which do not give any adsorption of this antibody. The antibody has been shown to have some specificity. (Hill & Haberman.)

The transfusion experiment (Fig. 4) suggests that sensitized red cells with globulin adsorbed on their surface are also removed rapidly from the circulation of a normal subject. The rapid fall in the number of transfused red cells simultaneously with the disappearance of the sensitized cells (Fig. 5), speaks strongly in favour of this assumption. The red cells which survived a normal time in this expriment (Fig. 4) may have been unsensitized at the time of transfusion, or it may perhaps be possible that if weakly sensitized, they may have become desensitized in a normal circulation. Loutit (1946) and Loutit & Mollison (1946) in similar expriments have found for the most part normal survival times for sensitized red cells transfused to a normal sub-

ject. In their cases the transfused red cells were not followed up by the Coombs developing test, and it is uncertain whether the cells survived as sensitized cells or whether they became desensitized in the normal subject. The discrepancy between the results seems to be due to varying degrees of sensitization. Such a variation is obvious in patients suffering from acquired hemolytic jaundice, the rate of destruction varying considerably. The case reported here, showed very strong sensitization as judged by the anti-human-globulin test, and the rapid rate of destruction.

The cause of the formation of an antibody against the red cells in acquired hemolytic jaundice is still unknown. In view of the known formation of hemagglutinins in various virus infections, it would be worth while considering the suggestion of Hayem in 1898 when he characterized the disease as "ictere infectieux chronique splenomegalique". Whether infection plays a part or not the disease is resistent to treatment with penicillin.

CONCLUSION

The data reported indicate that certain cases of acquired hemolytic jaundice are due to the formation of an immune antibody against the red cells, and that the antibody is adsorbed on the surface of the red cells, giving a positive Coomb's developing test, and altering the cell in such a way that it is removed from the circulation, probably by a normal reticuloendothelial system.

SUMMARY

A case of acquired hemolytic jaundice is reported in a man aged 74 presenting the following characteristics: Jaundice, anemia, splenomegaly, reticulocytosis, spherocytosis and increased fragility of the red cells. The red cells showed clumping in the patients own serum and were strongly agglutinated when exposed to anti-human-globulin rabbit serum. The blood stream in the conjunctival vessels showed "sludged" blood.

Normal red cells transfused to the patient were shown by means of the anti-human-globulin serum to be sensitized, and estimation of the survival time revealed that they were removed rapidly from the circulation.

After transfusion of red cells from the patient to a normal subject, the sensitized cells, both those of the patient and those which he had previously received by transfusion disappeared from the circulation of the normal recipient in a few days. The remainder of the transfused cells, being unsensitized as checked by the anti-human-globulin test, survived a normal time.

The rate of destruction of the red cells in the patient was found to correspond to about $\frac{1}{2}$ litre per day. Heparin in large doses seemed to impede the rate of destruction.

The findings support the view that this type of acquired hemolytic jaundice is caused by the formation of an immune antibody which is adsorbed on the surface of the red cells. Such sensitized cells are also removed from the circulation in normal individuals, probably by the reticulo-endothelial system.

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THE ENDOCELLULAR NUCLEIC ACID DISTRIBUTION
AND PLASMA PROTEIN FORMATION IN MYELOMATOSIS

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Multiple myeloma is a disease of unitary cell origin (Wallgren 1920). The theory advanced by Apitz in a series of publications from 1937 to 1940, that myeloma is a neoplastic proliferation of the plasma cells, has now been widely accepted (for a rceent review, see Bayrd 1948). The bone marrow from myeloma patients is found to contain these plasma cell elements in all stages of maturation. The least differentiated of them appear to be what are known as typical myeloma cells.

In 60 per cent or more of the myeloma cases an increase in the plasma proteins can be observed. Through fractionated precipitation with neutral salts it early was made clear that different globulin fractions could be the cause of the hyperproteinemia. However, it was through the electrophoretic separation method and the ultracentrifugal analysis that we obtained clearer conception of the variety and the extent of the disturbances in the composition of the blood proteins that appear in myeloma cases.

Longsworth, Shedlovsky and Mac Innes (1939) were the first to publish the results of an electrophoretic analysis of myeloma serums: two of the cases displayed β-globulin increase, the third was apparently normal. Kekwick (1940) investigated 5 cases electrophoretically and ultracentrifugally and he found in 4 serums γ-globulin enhancement with sedimentation constants corresponding to normal γ-globulin. The fifth case exhibited an increase of the β-component which was electrophoretically homogeneous. The ultracentrifugal analysis, however, showed 4 abnormal β-components. Hence not only quantitative displacements in the globulins are to be found but even the new formation of abnormal proteins, (in addition to the previously known Bence Jones' protein) has been certified. Gutman and coworkers (1941) added the observation that even electrophoretically certain myeloma serums present an abnormal component, termed M, migrating between the β and γ globulins. These authors were of the opinion that the M peak in the diagram most likely corresponded to the Bence Jones' protein in the serum, as the urine from the patients contained a component with the solubility properties of B. J. protein; furthermore, there appeared an extra peak corresponding to M in the diagram of normal serum to which was added the urinary protein from these

4

cases. Later on, however, Moore, Kabat and Gutman (1943) showed through molecular weight analyses that the M component is not always identical with the B. J. protein. In general, this latter cannot be detected electrophoretically in the serum, despite its presence in the urine, for which reason these cases sometimes display normal serum patterns.

The electrophoretic serum findings in myeloma are later confirmed by several investigators (Olhagen 1944, 1947, Wuhrmann and Wunderly 1945, Malmros and Blix 1946 and others). It has been unanimously stated that the the γ type represents the most frequently occurring form of hyperglobulinemia in myeloma. Yet it has not been made clear whether the globulin is only quantitatively changed or even qualitatively altered. In the majority of cases both the mobility and the molecular weight seem to correspond with those of normal γ-globulin. Olhagen (1945, 1947) showed however that certain cases of myeloma exhibit an abnormal γ-globulin with faster mobility and greater molecular weight than the normal γ-globulin. This pathologic globulin, termed γ_x, shows pronounced anticomplementary qualities after heating, and the inactivated serum causes auto-inhibition in the Wassermann reaction.

The relationship between the myeloma cells and the increase in serum proteins has long been the subject of disucssion. Most people assume that the myeloma cells are responsible for the new-formation of the various proteins (see Lichtenstein and Jaffe 1947, p. 223) ; others, however, advocate the opinion that they play a passive role as depots for the protein produced at some

other place (see Devine 1941, Waldenström 1942).

This study of multiple myeloma is primarily concerned with the question of whether or not the myeloma cells can be regarded, from a cytochemical point of view, as producers of the pathological hyperproteinemia. Subsequently we will find out, if there is any correlation between the cytochemistry of the myeloma cells and the type of protein eventually produced by them.

The starting-point for the investigation is the last ten years experience on the rôle of nucleic acids in the cellular protein formation. It has been shown (Caspersson and collaborators 1939—1945, Brachet 1941—1947 and others) that an intense protein production of a cell always occurs in the presence of pentose nucleic acids in the cytoplasm and nucleolus. Although the underlying mechanism is still unknown, this fact seems to be of general validity.

The main method for cellular nucleic acid determination has been ultraviolet microscopy combined with quantitative photometrical procedures. With such methods Thorell and Wising 1944 found that the myeloma cell in general had the cytochemical characteristics of an intensely protein-producing cell: large concentrations of pentose nucleic acids in the cytoplasm, and also a nucleolus containing pentose nucleic acids. The myeloma cell was thus found to have a cytochemical organisation similar to that of the growing blood stem cell (Thorell 1944, 1947) or the protein-secreting gland cell (Caspersson, Hydén and Aquilonius 1941). This seemed to be in accordance with the view that the myeloma

cells are the producers of the increased serum protein components.

Lichtenstein and Jaffe 1947, in an investigation of 35 cases, could divide the myeloma cells into two main groups from a cytological point of view. This cytological variation, they believed, reflected stages in the maturation of the basic tumor cell. Essentially the same conclusion was made by Bayrd 1948, who also could show that the differential cytological picture bore a rather significant relationship to the duration of the disease as expected from the degree of differentiation of the myeloma tumor cells. But probably due to a lack of proper definition of the different types of myeloma cells and also to incomplete corresponding protein analyses, one has hitherto failed in correlating a certain type of cell with a particular kind of protein. In the following, we will see if the cytochemistry of the myeloma cells compared with protein analyses can give any informations about this.

MATERIAL

The present investigation is based on material from sixteen clinically typical cases of multiple myeloma. In all cases the sternal marrow punctate (stained according to Giemsa) showed the picture typical for this disease. All cases but two presented multiple osteoclastic foci of characteristic appearance on X-ray examination of the skeleton. The two cases (nr. 4 and 16) without visible roentgenological alterations of the bone system both showed a considerable and significant γ-globulin increase. Case nr. 4, moreover, was verified post mortem.

METHODS FOR THE CYTOCHEMICAL ANALYSES

In order to examine roughly the distribution of nucleic acids in the individual myeloma cells, ultraviolet-microphotographs were taken of living cells obtained by sternal puncture. The wavelength was 2570 Å, thus very near the absorption maximum of the nucleic acids. The ultraviolet microscope constructed by Köhler was used. As the nucleic acids dominate the light absorption at this wavelength, the parts rich in these substances appear on the microphotograph as dark areas (Caspersson 1936).

As a qualitative test for desoxypentose nucleic acid the Feulgen reaction was adopted (Feulgen and Rossenbeck 1924). It was performed on fixed smear preparations of myeloma cells.

In order to obtain quantitative ultraviolet absorption data the photographic-photometric technique described by Thorell (1947) was used. Complete absorption spectra in the wavelength range 3100—2400 Å of points in the cytoplasm of intact myeloma cells were taken by this method. Also the intracellular distribution of absorbing substances at 2570 Å was determined photometrically in a similar way.

METHODS FOR THE PROTEIN ANALYSES

The total protein content of serum of heparinized plasma was determined according to the micro-Kjelldahl method. The electrophoretic analyses were performed with the Tiselius—Svensson apparatus in accordance with the principles laid down by Svensson (1943) and worked out by Olhagen (1945). The buffer employed was 0.032 mol. Na_2HPO_4 + 0.004 mol. NaH_2PO_4 + 0.15 mol. NaCl, pH 7.6. Table I reveals the limit of variation of the normal plasma components (Olhagen 1947). The urine was repeatedly tested chemically for the presence of Bence Jones' protein, and the cases with proteinuria were analyzed electrophoretically.

Table I.

No.	Case and no. of record	Date	Total protein g/100 ml	Electrophoretic analysis; relative conc. in per cent					Bence Jones' proteinuria	Myeloma-celltype	Remarks.
				alb.	α	β	φ	γ			
1	K. N. (M. 897/42)	6/11 −42	12.4	22.7	3 6	9.7	6.5	57.5	·0	A	
2	J. O. (M. 1635/42)	6/11 −42	12·6	26.7	7.5	7.5	—	58.3	o	A	γx globulin.
3	R. N. (M. 1961/43)	18/10 −43	9.3	41.2	6.0	42.8	3.1	6.9	o	A	
4	E. L. (M. 378/44)	8/3 −44	8.1	32 9	6.6	15.9	8.8	35.8	o	A	+atypical cells. Par- amyloidosis.
5	K. A. (Rh 9584/44)	25/5 −44	10.7	15.1	5.5	7.0	4.5	67.9	o	A	
7	A. G. (M. 826/44)	17/5 −45	11.9	24.0	5.6	8.2	—	60.1	o	A	γx globulin.
9	O. W. (M. 2252/45)	15/11 −45	12.1	18.8	6 6	8.2	5.1	61.3	o	A	+B?
10	E. A. (Rh. 821/46)	19/2 −46	11.2	18.5	8.1	7.3	—	66.1	o	A	
11	R. J. (M. 1650/46)	19/9 −46	9.1	27.6	4.1	9.2	3.6	55.5	+	A + B	Preponderatingly A cells.
13	M. B. (S. E. 7091/46)	6/12 −46	10.1	32.2	6.3	7.9	—	53.6	+	A + B	Preponderatingly A cells.
16	E. K. (M. 618/47)	9/4 −47	8.2	50.2	5.7	14.1	—	30.0	o	A	+B?
6	E. J. (Rh. 14322/44)	4/11 −44	6.6	53.3	8.7	17.8	—	20.2	+	B	
8	H. W. (M. 1539/45)	15/10 −45	6.8	45.9	8.3	14.6	7 6	23.6	+	A + B	+atypical cells. Par- amyloidosis.
12	G. S. (Rh. 14966/46)	23.9 −46	7.6	51.9	9.3	14.1	8.8	15.9	+	B	
14	I. A. (M. 257/47)	4/2 −47	6.7	54.3	11.6	15.9	4.2	14.0	o	B	+reticuloid cells (do- minating).
14	I. A. (M. 257/47)	14/5 −47	6.1	46.5	7.6	23.1	8 5	14.3	+	B	+reticuloid cells (sparsely).
15	S. H. (M. 1168/47)	29/3 −47	7.5	59.8	4.7	21.5	—	14.0	+	B	+reticuloid cells (sparsely).

Table II.

		Alb	α	β	φ	γ
A	Serum-average (20 cases) ...	58.2	7.2	14.5	—	20.1
	Normal range (± 2σ)	53.2 − 63.2	5.2--9.2	11.3 − 17.7	—	15.9 − 24.3
B	Plasma-average (10 cases) ...	56.2	7.0	14.0	4.0	18.8
	Normal range (± 2σ)	50.8 − 61 6	4.8 − 9.2	9 8 − 18.2	3.0 − 5.0	15.4 − 22.2

RESULTS OF THE CYTOCHEMICAL ANALYSES

It was obvious after investigation of the very first cases that the myeloma cells could be divided in two main groups. These can be described as follows:

1. Case I, II, III, IV, V, VII, VIII, IX, X, XI, XIII and XVI (see Table II). Cells with relatively small nuclear-cyto-plasmic ratio. The cytoplasmic nucleic acid concentration as calculated from the absorption measurements (Fig. 1) is around 3 per cent. These nucleic acids are Feulgen nega-tive and can thus be regarded as of pentose type. They are distributed in the cytoplasm as characteristic for mature plasma cells (Fig. 2), i. e. the highest concentration peri-
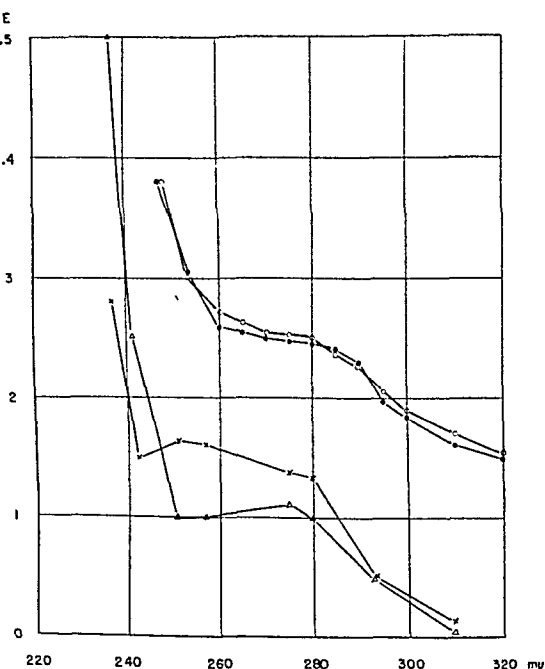
Fig. 1. Absorption spectra of points in the cytoplasm of A-type myeloma cells. The two upper curves are from a fixed preparation (formol-acetic acid). Using the thickness-determinations of the cells at measured points, the average nucleic acid concentration can be calculated to about 3 per cent. (Cases V, IX and XI.)

pherally (see Bing, Fagraeus and Thorell 1945).

These cells have in the nuclei large ultra-violet-absorbing, Feulgen negative nucleoli which are surrounded by a thick outer layer of Feulgen positive nucleolus-associated chromatin (Fig. 3). For the rest the desoxy-pentose-nucleic acid containing chromatin is evenly distributed as grains about 1μ in diameter.

As compared with earlier analyses of plasma-cells (see l. c.) the nucleic acid distri-bution in this type of myeloma cell corre-sponds to that in a mature plasma cell, with the exception that the pentose nucleic acid

Fig 2. Distribution of ultraviolet absorbing sub-stances at 2570 Å in the cytoplasm of an A-type myeloma cell. The concentration of absorbing substances (mainly nucleic acids) is highest peri-pherally. (Case X.)
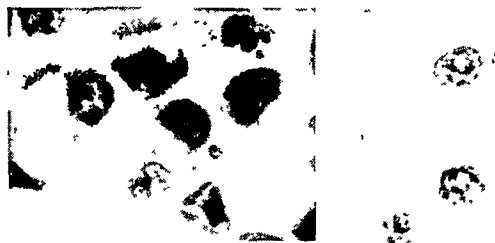
Fig. 3. a) Ultraviolet microphotograph taken at 2570 Å of intact A-type myeloma cells in physio-logical saline solution Objective: quartz mono-chromat 1.6 mm 1500 X enlargement. (Case X.) b) Myeloma cells stained with the Feulgen reac-tion. Note the Feulgen-negative nucleolus sur-rounded by Feulgen-positive nucleolus-associated chromatin. (Case III.)

containing parts of the nuclei (mainly the Feulgen negative nucleolus) are much increased.

In the discussion below this type of myeloma cell is called type A.

2. Case VI, VIII, XI, XII, XIII, XIV and XV (see Table II). Cells with a large nuclear-cytoplasmic ratio. In the cytoplasm there are relatively high concentrations of nucleic acids (5 %), which are of the pentose type (Fig. 4). These nucleic acids are
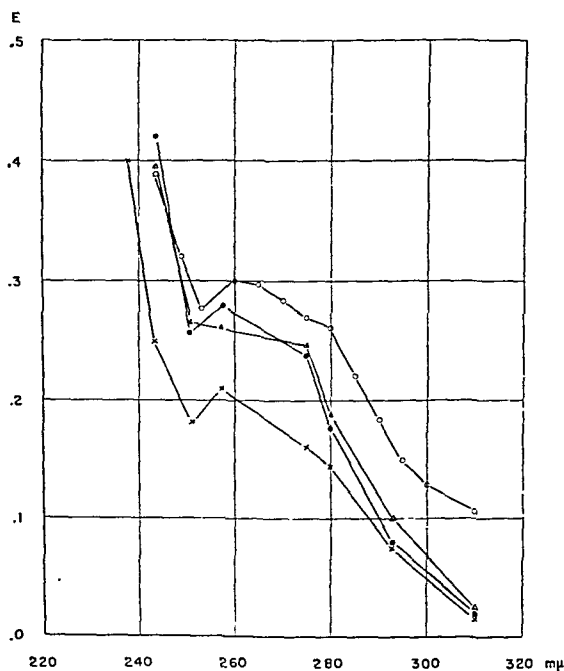
Fig. 4. Absorption spectra of points in the cytoplasm of B-type myeloma cells. The average nucleic acid concentration can be calculated to about 5 percent. (Case VI and XIV.)

evenly distributed from the nuclear membrane out to the periphery of the cell (Fig. 5).

The desoxypentose nucleic acid containing chromatin is sparse and evenly distributed,
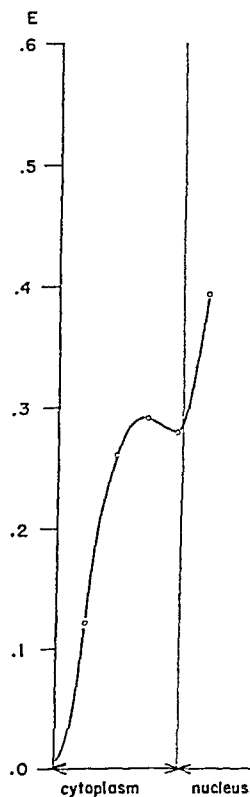
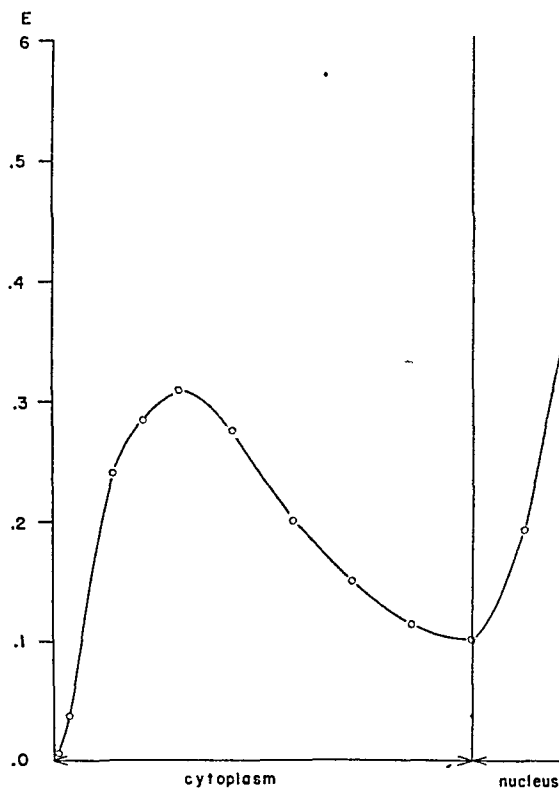Fig. 5. Distribution of ultraviolet-absorbing substances at 2570 Å in the cytoplasm of a B-type myeloma cell. The absorbing substances are evenly distributed in the cytoplasm. (Case XV.)

except for the formation of an outer layer around the Feulgen negative nucleolus. The latter usually extremely large.

In certain cases there can be seen transformations of this type of cell into necrotic forms, i. e. cells with remaining morphological organization but diminishing concentrations of nucleic acids (Fig. 6).

This type of myeloma cell is, contrary to the plasmacell-like A-type, more similar to a malignant, undifferentiated cell. It is in the discussion below called myeloma cell type B.

Fig. 6 a.
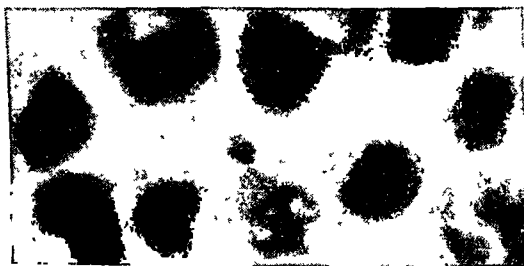
Fig. 6 b.
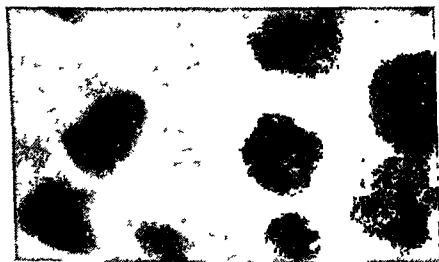
Fig. 6 c.

Fig. 6 a) Ultraviolet microphotograph taken at 2570 Å of intact B-type myeloma cells in physiological saline solution. (Case VI.) Obj.: quartz monochr. 1 6 mm 1500 X enlargement   b) Ultraviolet microphotograph of myeloma cells from case XIII with both A and B type   Same data as above   c) Ultraviolet microphotograph of two necrotic B-cells (in the middle of the figure). Same data as above   (Case VI)

As can be seen from Table II some of the cases exhibit both types of cells (Nos. VIII, XI and XIII).

RESULTS OF THE PROTEIN ANALYSES

From an electrophoretic point of view the material can be divided in two groups:

1. Cases with pronounced globulin enhancement. About two thirds of the material, i. e. 11 cases, belong to this group. The hyperglobulinemia is statistically significant, leading to hyperproteinemia in 9 cases. Only case no. III shows β-globulin increase, all the others are of the γ type. (Table II).

2. Cases with very small changes of the globulin part and with total serum protein values within the normal range. This group comprises the remaining 5 cases. (Table II).

Bence Jones' protein was found in the urine of 7 cases, i. e. 44 per cent. Strikingly all the cases belonging to group no. 2 are distinguished by the presence of this protein, which was electrophoretically characterized by mobility values in the region of β and γ-globulin. It is rather tempting to imagine that the slight increase of the β-component in the cases no. XIV and no. XV, perhaps even no. VI were a sign of Bence Jones' proteinemia. No attempts to prove this assumption have been made, however.

With respect to the electrophoretic serum pattern it is interesting to note that all cases with γ-globulin increase differ qualitatively from other hyper-γ-globulinemic sera, for example liver cirrhosis and lupus erythematosus disseminatus. As can be seen from Fig. 7 the γ-globulin peak in myeloma presents an abnormally narrow configuration, which is due to the differences in boundary spreading of the myeloma globulin and other γ-globulins. This phenomenon has its mathematical expression in the heterogeneity constant (Sharp, Cooper and Neu-
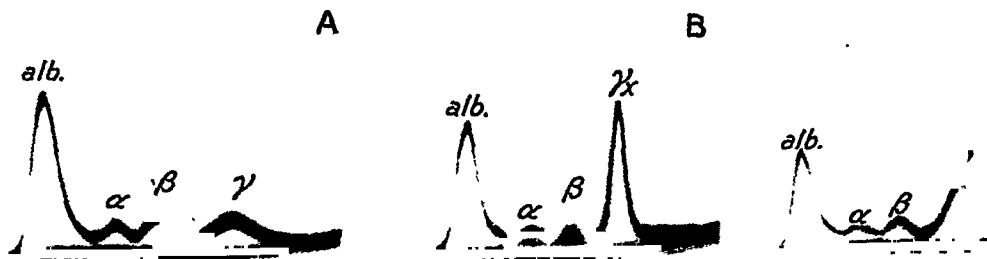
Fig. 7. Electrophoretic serum patterns. A. Normal. B. Myeloma. Note the abnormal configuration the γ peak.   C. Liver cirrhosis with γ globulin increase of the same magnitude as serum B.

rath 1942) and has been calculated for isolated γ-globulins (Olhagen 1945) as well as for the whole γ-component in the serum diagram (to be published).

SURVEY AND DISCUSSION OF THE RESULTS

This investigation firstly shows that the typical bone marrow cells in myelomatosis are cytochemically characterized by pentose nucleic acids in the cytoplasm and a well developed nucleolar apparatus, containing pentose nucleic acids. Earlier experience has shown that this is the pattern of protein producing cells, for example the growing blood stem cell or the protein secreting gland cell. It is not possible, however, on the basis of the present experimental data, to determine whether this protein produced by the myeloma cells is used for the building-up of the growing cell itself, or for the production of a protein-containing secretion. In the cases of myelomatosis reported above, which exhibit pathologically increased serum globulins or Bence Jones' protein, it is, on the other hand, very probable that the myeloma cells are responsible for the production of these proteins.

The myeloma cells have been divided i two classes according to the distribution a amount of nucleic acids in them. If celltype is compared with the correspondi serum protein analyses (see Table II), it evident that the cases with type A cells ha a marked β or γ-globulin increase while cases with normal serum patterns have ty B cells. All the latter cases display Ber Jones' protein in the urine and consequen B. J. proteinemia, though not electi phoretically demonstrable. The cases (VI XI and XIII) which have both A and types of myeloma cells have both increas serum globulins and Bence Jones' protei

This correspondence indicates that t production of a special type of protein myelomatosis can be correlated with a par cular distribution and concentration of pe tose nucleic acids in the cell. The differe cytochemical organizations of the two typ of myeloma cell thus seem to be the fun tional bases for the presence of differe types of protein.

In this connection it is of interest to reca the endocellular nucleic acid distribution i the plasma cell during its development in tl course of an immunization (Bing, Fagraei

and Thorell 1945, Fagareus 1948). During the first stage of the immunization, the stage of incubation, the most frequently occurring type of cell was found to correspond, in cyto-chemical organization, to a rapidly growing cell, for example the blood stem cell. Thus it had a well developed nucleolar-apparatus and high concentrations of pentose nucleic acids in the cytoplasm. Mitoses were also found. During the later stage of the immunization, with the constantly high titre (and globulin-)level, a type of cell, identical with the mature plasma cell predominated. During the transition from the type of cell engaged in rapid growth to the later mature plasma cell, the changes in the nucleolar apparatus were the same as those observed during the blood cell maturation (see Thorell 1947) i. e. a rapid disappearance of the pentose nucleic acid containing nucleolus. However, the cytoplasmic nucleic acid concentration, measured as $E_{2573 Å}$, remained approximately unchanged during this development. These facts favoured the view that the plasmacell was functionally differentiated and no longer growing, and that its function was antibody protein production.

The A type of myeloma cell has a similar distribution of pentose nucleic acids as the mature plasma cell, except that the pentose nucleic acid containing nucleolar apparatus is well developed. We interpret the A-type of myeloma cell, as cytochemically defined above, to be a plasma cell with a disturbance in the nucleic acid metabolism resulting in increased growth and function.

The B-type of myeloma cell, on the other hand, has the characteristics of malignant growth. We interprete the cytochemical data of this type of cell to be an expression
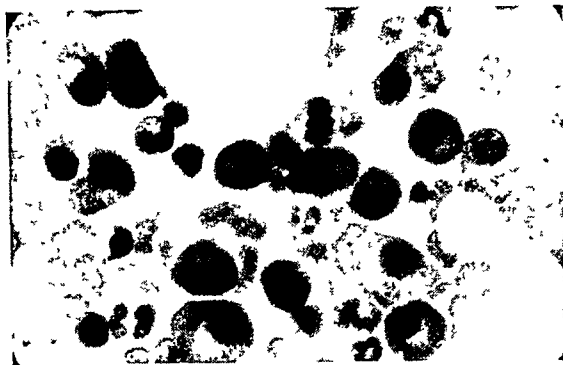
Fig. 8 a.
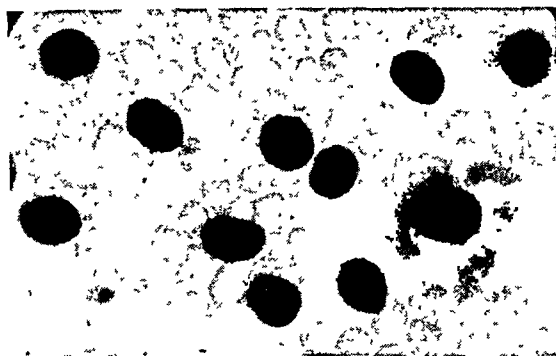
Fig 8 b.

Fig. 8. a) Giemsa-stained A-type myeloma cells. (Case X.) b) Giemsa-stained B-type myeloma cells (Case VI.)

of a disturbance in the nucleic acid metabolism resulting in intense growth of un-differentiated elements.

This may indicate that the production of a higher molecular weight protein (γ-globulin m w. > 150 000) requires a more highly differentiated cell organization (A-type) than does the production of one with a lower molecular weight (the B. J. protein weighing about 35 000; B-type of myeloma cell). Furthermore, it would seem that the myeloma cell mainly produces *abnormal* proteins. This assumption is supported by the

following facts: the frequent formation of B. J. protein, the aforementioned abnormal boundary spreading in myeloma serum of γ-type, the anticomplementary qualities of certain myeloma globulins, the demonstration of ultracentrifugally abnormal globulins (Kekwick l. c.) and the occasional finding of spontaneously crystallizing globulins.

The characteristics of these two types of myeloma cells is of course more or less re-flected in the cytological pictures as seen in an ordinary stained preparation (Fig. 8). The similarity of the A-type to plasma cell is for the most part evident, as is also the "myeloblastic" picture of the B-type. How-ever, we have found that this is not always the case, probably due to the flattening out of the cells in smear preparations and to the lack of reproduceability in the staining procedure.

SUMMARY

Myeloma cells from 16 typical cases have been analyzed with microspectrophotometric methods with regard to nucleic acid distri-bution and concentration. The blood serum proteins have been analyzed electrophore-tically and the urinary protein was also analyzed in the Tiselius' apparatus when present.

On the basis of the cytochemical analyses the myeloma cells can be divided into two distinct types; the "plasma cell like" A-type and the "myeloblastic" B-type.

Each type of myeloma cell can be corre-lated to a particular protein; the A-type with β or γ-serum globulin and the B-type with Bence-Jones' protein.

The data indicate that the myeloma cells are responsible for the pathological protein production in myelomatosis.

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THE FLUORIMETRIC DETERMINATION OF ADRENALIN IN BLOOD PLASMA

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Numerous chemical and physiological methods have been suggested for the determination of adrenaline in blood and plasma. Very variable adrenaline values in blood have in the past been obtained by different authors, but only recently have investigations contributed to the solution of the problem, this depending on the application of the fluorescence reaction of adrenaline from irradiation with ultraviolet light. Loewi (1918) and later Paget (1937) made the observation that an alkaline solution of adrenaline produces a green fluorescence when irradiated by ultraviolet light. In 1934 a more detailed study of this reaction was carried out by Gaddum and Schild who succeeded in determining as low concentrations as 0.01 μg per ml in aqueous solutions. Owing to a disturbing blue fluorescence from the plasma proteins, they had no success in determining the adrenaline content in blood with this method.

Different ways have been tried to eliminate the disturbing fluorescence of the proteins. Either the proteins have been removed by means of precipitation, e. g. by trichloracetic acid (Kobro, 1946) and the filtrate has been analysed, or blood has been dialysed

and the adrenaline determined in the prote free dialysate (Hueber, 1940; Kalaja Savolainen, 1941; Jørgensen, 1945; W(1947). The first of the above methods I proved to be impracticable, as big quantit of adrenaline are removed by the pre pitation of protein (Lehmann & Michae 1942). Opinions reg. the method of dialy have varied. Jørgensen (1945) and W((1947) however, have recenlty found means of a modification of this method tl the normal value for adrenaline in plasma about 0.07 μg per ml.

In 1942 Lehmann & Michaelis work out quite a different principle to remove t fluorescence of the proteins. These autho analyse serum or plasma directly a measure the fluorescence by means of Pulfrich photometer, the disturbing fluor scence of the proteins being cut off by a filt glass that absorbs the blue light, but tran mits the green fluorescence. Reading taken against a fluorescent glasstandard. I this method considerably higher values from 1—3 μg per ml — were obtaine

The question, why these two modificatio of the fluorescence method give such varyir values, has been taken into closer investi

ation by us (Annersten, Grönwall & Köiw, 1949; Annersten, 1949). We are supporting the view that adrenaline exists partly in a free dialysable form and partly in a non dialysable form bound to the proteins; according to this view the difference can be principally explained.

The fluorescence methods, however, have recently been criticized. Bloch (1948) has criticized the method involving dialysis, especially Jørgensen's method, considering it as unreliable, inter alia owing to the existence of an inhibitory factor in the dialysate. The method of Lehmann and Michaelis has not been used generally, and it has likewise been criticized. One of us (S. A.) has tried to use this method, however, without any success, v. Porat (1946) states that he was not successful "to show adrenaline in plasma by means of this method". Staub & Klinger (1948), having likewise investigated this method, support the view: "Die Methode ist unspezifisch und gestattet nicht den Adrenalingehalt des Venenblutes zu erfassen."

For our adrenaline determinations we wanted to choose a method enabling analysis of both plasma (serum) and its dialysate. The method of Lehmann and Michaelis certainly conveyed the impression of being very complicated technically, although acceptable from the point of view of principle. We did not succeed in obtaining reproducible values when making readings by a Pulfrich photometer. It seemed possible, however, to obtain a more sensitive and objective reading by means of a photocell equipped with an amplifier.

Since having availed ourselves of a Beckman spectrophotometer furnished with a fluorescence accessory set, and having modi-fied the method in certan points, we have succeeded in obtaining adrenaline values in plasma which closely agree with those reported by Lehmann and Michaelis. With this instrument it is possible to measure with satisfactory accuracy even very weak fluorescence. It also enables to carry out rapid and objective measurements which is of great importance, for the fluorescence develops quickly and having passed a maximum, it again disappears. We also have carried out a more detailed study of the effect of different concentrations of sodium hydroxide on the fluorescence, of the stipulations for the mixing of plasma and alkali, and of the depression of the adrenaline fluorescence by formaldehyde; thus working out a modification of the method of Lehmann and Michaelis, which we have found to be relatively simple and accurate.

METHOD

a) Principle.

Adrenaline is determined in the blood-plasma, taken in a "protective solution" of sodium thiosulphate that prevents the oxidation of adrenaline. To the mixture of adrenaline or blood-plasma and sodium thiosulphate in the sample cell, 10 per cent sodium hydroxide is added, whereupon the sample cell is irradiated from below by ultraviolet light. The intensity of the fluorescence is read in the photometer at right angles to the entering light by means of a phototube and special filters so that while the sample is irradiated by a definite ultraviolet light, the phototube is reached mainly by the green fluorescent light. By the addition of formaldehyde the adrenaline fluorescence is prevented, leaving the fluorescence caused by the proteins undisturbed and a new reading indicates how much of the first reading corresponded to the adrenaline. From those two readings and a third one, carried out with a known adrenaline solution, the adrenaline content of the sample can be calculated.
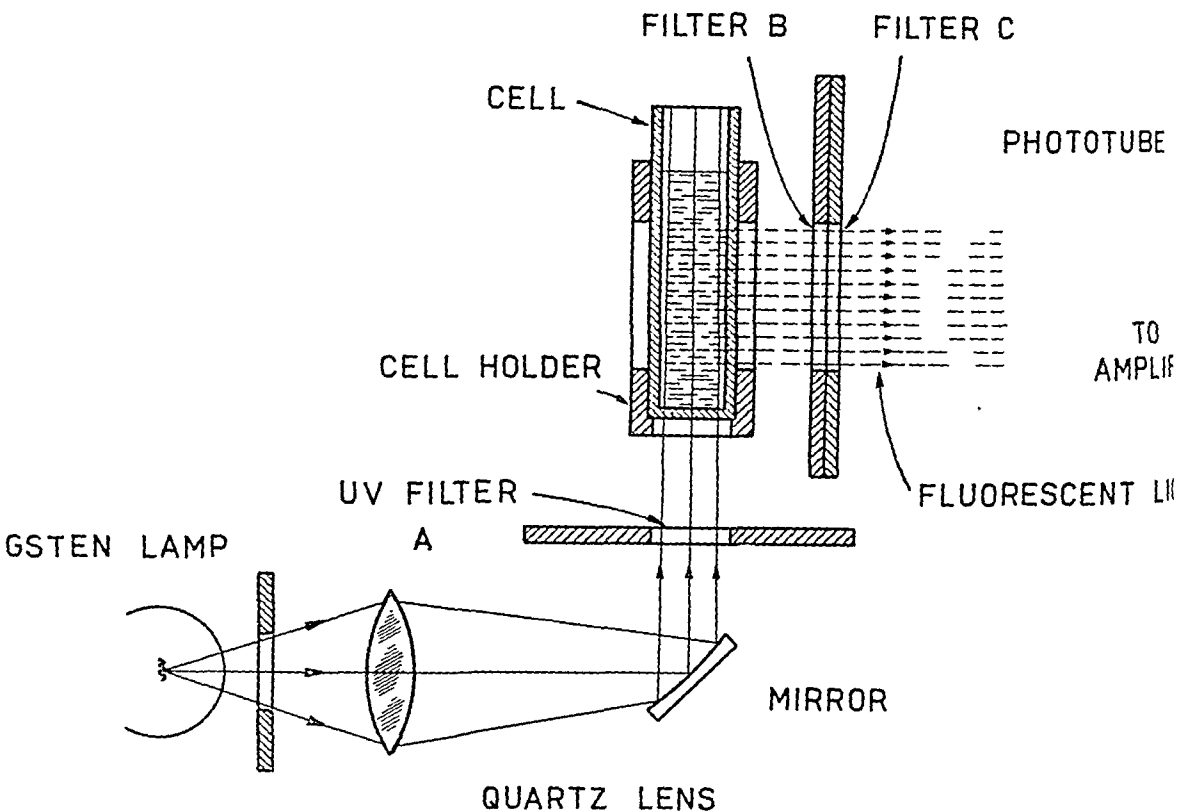
Fig. 1. Optical arrangement for determination of adrenaline.

b) Apparatus, other accessories and solutions.

The Beckman spectrophotometer with fluorescence accessory set and corex cells.

Micropipettes á 0.1 ml.

Pipettes 1 ml and 2 ml.

Pyrex glass-sticks for stirring and mixing of sample and sodium hydroxide in cells.

Graduated centrifuge tubes. ·

Sodium hydroxide, 10 per cent solution.

Formaldehyde, 40 per cent solution.

Adrenaline solution 50 μg per ml in 1.5 per cent sodium thiosulphate.

"Protective solution" for blood, 1 g sodium citrate and 3 g sodium thiosulphate in 100 ml redistilled water.

c) The Beckman spectrophotometer with fluorescence accessory set and optical arrangement for adrenaline analysis.

The Beckman quartz spectrophotometer with fluorescence accessory set makes it possible to measure even very weak fluorescences. This is reached, partly because the intensity of the irradiating ultraviolet light is so low that the photochemically sensitive compounds in the sample are not affected, and partly because the sensitiveness of the phototube is increased by the amplifier.

The course of the light-beams and the optical arrangement are indicated in Fig. 1. The ultraviolet light is focussed through a quartz-lens on a mirror, which reflects it at angle of 90 degrees upwards through an ultraviolet filter (A), by means of which only the ultraviolet light is permitted to pass, the visible light being absorbed (Fig. 2). The ultraviolet light then reaches the bottom of the cell (Fig. 1), producing a fluorescence in the sample solution. The fluorescence light passes filter B and filter C, reaches the photo-

Fig. 2. Transmission curve of filter A.
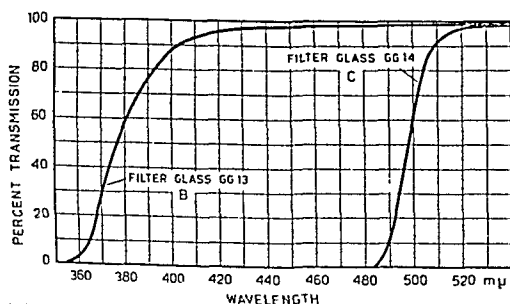
Fig. 3. Transmission curves of filters B and C.

tube, whereby the current is amplified and recorded by the galvanometer.

Filter C that is placed between the cell and the phototube, is a Schott GG 14 that transmits the green light, whereas most of the shortwave light under 490 mµ is cut off, and thus also the greatest part of the fluorescence from the proteins (Fig. 3). Filter C fluoresces in ultraviolet irradiation. In order not to disturb the reading, filter B is placed between the cell and filter C. Filter B is a Schott GG 13 that absorbs ultraviolet light, thus protecting filter C from that irradiation.
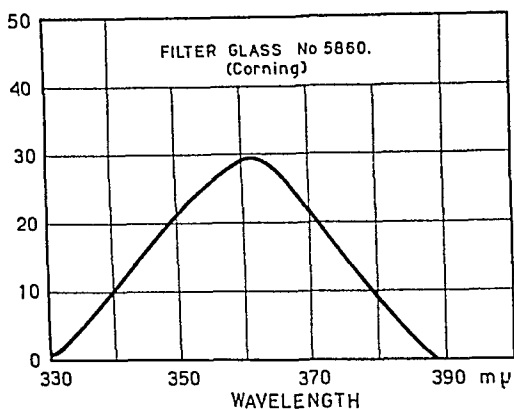
d) Determination of the adrenaline content in blood-plasma.

In a graduated pyrex test-tube 1.5 ml of the protective solution is transferred. By means of venous puncture 4.5 ml blood is taken directly into the protective solution. After centrifugation of the mixture, plasma is removed by a pipette, and shaken in a test-tube for about 5 minutes to

be saturated with oxygen at the tension of air. 1 ml of the mixture of the plasma-protective solution is transferred to the cell and mixed with 2 ml 10 per cent sodium hydroxide. This is carried out with the help of a pyrex glass-stick swiftly but carefully. Sodium hydroxide must not contain insoluble carbonate or any air-bubbles. About 15 vertical movements with the glass-stick are sufficient. The cell is placed as quickly as possible in the photometer for reading, and the maximum fluorescence is recorded (= reading I).

After the first reading 0.1 ml of 40 per cent formaldehyde is added, the stirring being carried out as described above, and the cell having been kept in darkness for 5 minutes (placed in the photometer), the fluorescence is read anew. The formaldehyde has then destroyed the fluorescence of adrenaline, and only that part of the fluorescence of the proteins is read, which has passed filter C (= reading II).

0.1 ml of a fresh adrenaline standard solution, containing 50 µg/ml, is added to the contents of the cell; the stirring is carried out as formerly — with about eight movements, and the maximum fluorescence is recorded (= reading III) .

It greatly adds to the complicacy of the calculation that the sample fluorescence and that of the standard solution are not measured under identical conditions. In the first case (reading I), reading is made while formaldehyde is absent. In the following case (reading III) measuring is carried out in presence of formaldehyde that had enabled reading II. Whereas the fluorescence is depressed by the formaldehyde, reading III results in a considerably lower value than it ought to have been in a fromaldehyde-free solution. Owing to this, it is necessary to know the relation between the intensities of the adrenaline fluorescence in the absence and presence of formaldehyde. In conformity with Lehmann and Michaelis we have also experimentally established the factor by which the quotient

$$\frac{\text{fluorescence of unknown solution}}{\text{fluorescence of known solution}}$$

or

$$\frac{\text{reading I} - \text{reading II}}{\text{reading III} - \text{reading II}}$$

has to be multiplied to obtain the adrenaline concentration, and have found it to be equal to 1.36 when blood is diluted according to the direction given above, and 0.85 in sodium thiosulphate solution.

Example:

Reading   I 34.8 scale units
   »   II 26.1 »   »
   »   III 30.2 »   »

$$\frac{(34.8 - 26.1)}{(30.2 - 26.1)} \times 1.36 = 2.9 \text{ } \mu\text{g. per ml}$$

DISCUSSION

Here we should like to discuss some important points concerning our procedure, control-tests and certain other results:

a) Development of adrenaline fluorescence by sodium hydroxide.

The adrenaline fluorescence, which is green with a maximum of 496 mμ, is not constant; after sodium hydroxide having been added, it develops, reaches a maximum, whereupon it disappears again. The higher the concentration of alkali, the higher the maximum, and the more quickly it is reached. The time for reaching the maximum also depends on the adrenaline concentration, being inversely proportional. An adjustment of the alkali concentration is of importance to facilitate easier reading. We have established that in low adrenaline concentrations, and by adding 2.5 N (10 per cent) sodium hydroxide the maximum is reached in 1.5—2.5 minutes, which gives satisfactory conditions for readings. We have found it to be of importance that the mixing of sodium hydroxide and plasma is carried out with a pyrex glass-stick quickly and carefully. Its lower part is curved, and while stirring it must not be lifted above the surface of the fluid to prevent air-bubbles

form entering the solution. It is necessary that close attention be paid to this each time. The stirring is carried out with equal and swift vertical movements.

b) Adrenaline for the purpose of standardization and standard solutions.

We have obtained for the purpose of standardization of the method and as comparison solution when carrying out analysis a commercial adrenalin-product "Original Adrenalin" delivered in a concentration of 1 : 1000 by Parke, Davis and Co., Detroit, U. S. A. Suitable solutions e. g. the standard solution containing 50 μg per ml, are prepared of it by dilution with 1.5 per cent sodium thiosulphate solution of the highest purity.

We have tried to use even other commercial products, but with varying success. Thus certain cases have proved that reading III gave the same or only a insignificantly higher value than reading II. This can probably be explained by varying sensitiveness of the adrenaline products to formaldehyde. We wish to point this out especially as it could possibly be one of the causes for the divergent results of some writers (v. Porat, 1946; Bloch, 1948; Staub & Klinger, 1948). In Fig. 4 the curves show the relative fluorescence of water solutions of different adrenaline products. It is obvious that the Swedish products give a higher fluorescence than the adrenaline manufactured by Parke, Davis and Co., Detroit. These variations in fluorescence of different products of adrenaline and the effect of formaldehyde on them do not make them unsuitable as standards in this method. It is, however, necessary to consider these properties when deciding the concentration of the standard solution. In any case it is important to carry out all
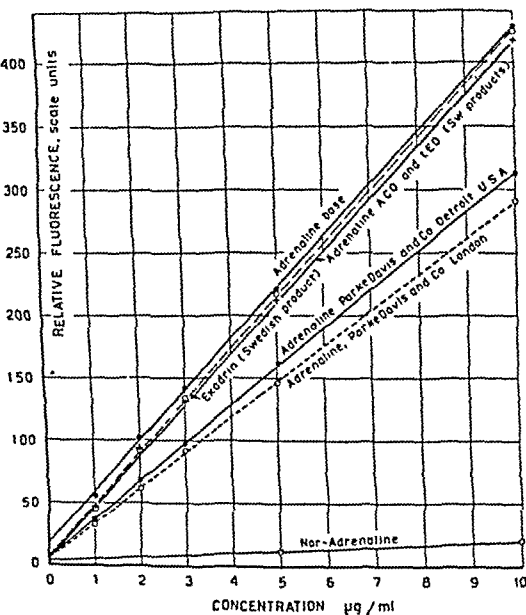
Fig. 4. Relative fluorescence of different adrenaline preparations.

determinations with the same adrenaline product as a standard, and it seems to us most convenient to store it in a solid state and in glass ampoules.

c) Specificity of the adrenaline fluorescence.

The reliability of the method is entirely due to the fluorescence being specific to the adrenaline. Besides adrenaline even other substances fluoresce when irradiated by ultraviolet light. Their influence, however, is reduced by the arrangement of suitable filters as described above. Besides adrenaline no other substances with the same fluorescence are known. Chemical compounds closely related to adrenaline, however, can show a similar fluorescence. This applies e. g. to noradrenaline, which was investigated by West (1947). With this substance a maximum fluorescence is also reached, but

considerably more slowly, and only at higher concentration. It appears by no means improbable that a small part of the adrenaline fluorescence, measured by this method, can be produced by noradrenaline and compounds closely related. Further investigations regarding the fluorescence of adrenaline and of other closely related substances are therefore desirable.

d) Addition of formaldehyde.

In addition to the fluorescence the method is based on the fact that the former is selectively destroyed by formaldehyde. By taking readings — before — and a certain time after the addition of formaldehyde, the fluorescence produced by the adrenaline content of the sample can be calculated. A solution of known adrenaline concentration is added to the sample when it subsequently contains formaldehyde. The fluorescence developed is weaker than it would have been in an aqueous solution. The behaviour of the adrenaline fluorescence in presence of formaldehyde has not been investigated formerly, and therefore we have carried out some experiments to elucidate this question. In Table I the fluorescence figures are given for 50 μg of different adrenaline products in 3 ml sodium hydroxide in presence of 0.1 ml 40 per cent formaldehyde. The effect of formaldehyde on the fluorescence is in all cases depressing, but it is not so great in the case of the american (Parke, Davis and Co.) adrenaline as in case of the other products.

e) Determination of adrenaline in known aqueous solutions, and in plasma with an addition of known quantity of adrenaline.

Lehmann & Michaelis (1942) have established that the maximum of adrenaline fluo-

Table I.

The Relative Fluorescence of 50 µg per ml. Adrenaline in Aqueous Solution and 10 per cent Sodium Hydroxide in Presence of 0.1 ml. Formaldehyde.

Rel. fluor. \ Adrenaline product	Parke, Davis and Co. Detroit, U. S. A.	Parke, Davis and Co, London	Exadrin, (Swedish product)	A C O Adrenaline (Swedish product)	Adrenaline base
Scale units	30.3	21.0	23.6	21.3	27.6
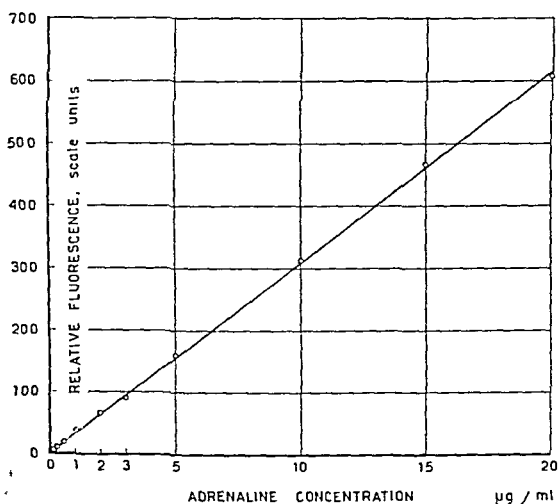
Fig. 5. Relative fluorescence of adrenaline at different concentrations.

Fig. 6. Determinations on plasma with a known quantity of adrenaline added.

rescence is proportional to a concentration up to 300 µg per ml (Lambert—Beer's law). The curve in Fig. 5 shows this relationship in different concentrations from 0—20 µg per ml. The curve does not pass zero, but the concentration being 0, a low blank value is obtained, which depends on the fluorescence of the cell and sodium hydroxide. It is evident that the dispersion of the values is small.

We have investigated the reliability of the method by tests for recovery of adre-naline by carrying out determinations on plasma; the adrenaline content of it was first determined, and then a known quantity of adrenaline added. See Fig. 6 and Table II.

We have also controlled the method in a number of cases by carrying out analysis of samples taken at the same time from the same patient's right and left arm. Corresponding results are shown by Fig. 7 and Table III.

Table II.

Date	Patient	Calculated content of adrenaline in μg per ml.	Obtained content of adrenaline in μg per ml.
3/3 − 48	22	5.00	4.83
3/3 − 48	23	6.85	6.70
3/3 − 48	23	6.90	6.78
4/3 − 48	24	5.81	5.69
4/3 − 48	24	5.95	5.57
6/3 − 48	25	5.19	5.20
8/3 − 48	26	5.14	5.20
9/3 − 48	27	5.36	5.18
12/3 − 48	28	6.86	6.78
13/3 − 48	29	6.25	6.40
15/3 − 48	30	6.31	6.44
15/3 − 48	30	7.05	7.30
16/3 − 48	31	5.57	5.49
17/3 − 48	32	6.95	7.05
17/3 − 48	33	5.82	5.75
18/3 − 48	34	6.05	6.12

Standard deviation (σ) = 0.13.   Variation coefficient (V) = 1.7.

Table III.

Date	Patient	Content of adrenaline in μg per ml. Left arm	Content of adrenaline in μg per ml. Right arm
1/3 − 48	29	1.13	1.17
1/3 − 48	29	1.22	1.24
2/3 − 48	23	3.81	3.60
4/3 − 48	29	1.58	1.64
6/3 − 48	30	0.39	0.34
6/3 − 48	30	0.43	0.45
6/3 − 48	35	0.96	0.91
6/3 − 48	27	4.04	4.10
8/3 − 48	30	0.27	0.24
9/3 − 48	36	0.14	0.13
12/3 − 48	29	1.58	1.44
13/3 − 48	37	2.66	2.50
13/3 − 48	38	2.42	2.48
15/3 − 48	39	2 80	2.62
15/3 − 48	39	2.71	2.58
16/3 − 48	40	1.60	1.54
16/3 − 48	41	1.36	1.48
17/3 − 48	42	2.13	2.06
18/3 − 48	38	2.50	2.42

Standard deviation (σ) = 0.07   Variation coefficient (V) = 3.9.

Fig. 7. Simultaneous determination of adrenaline on plasma from the right and the left cubital vein.

FREE AND BOUND ADRENALINE

The variable statements concerning the adrenaline content in blood probably are due to the different methods. It has been -stated that the biological methods result in values, whi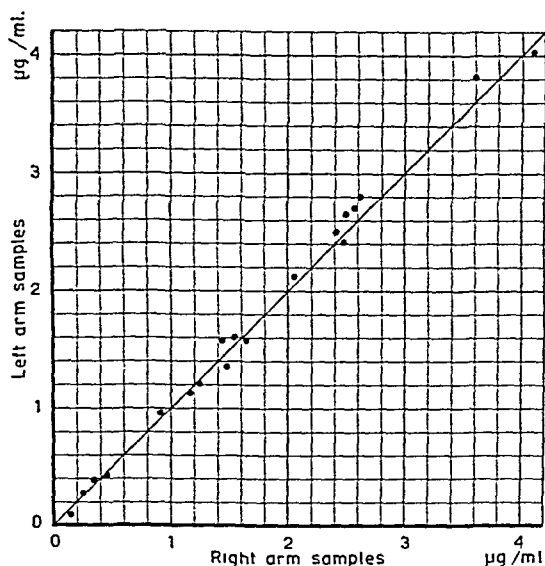ch are in every way lower than those obtained by the chemical methods. This fact has already earlier caused the belief that either a portion of the chemically determinable adrenaline is a biologically inactive preliminary stage of the active adrenaline or it is in a certain manner bound to the proteins with reduced or abolished biological activity.

By the method here described it is possible to determine adrenaline, both in the plasma, and in the dialysate of plasma. Due to this we have found sufficiently reasonable basis in this connection to discuss the problem, why such variable values are obtained by the dialysis method utilized by Hueber, Kalaja, Savolainen, Jørgensen and West compared with the method of Lehmann and Michaelis.

We have dialysed serum according to Jørgensen against a glycocoll-hydrochlorid acid buffer solution, and have carried out simultaneous analysis on serum and dialysate. After four hours we found constant values in the dialysate equal to those established by Jørgensen and West. In the serum we found a corresponding reduction, but the main part or 96—97 per cent did not pass the membrane, and is very likely

Table IV.

Exp. no.	Adrenaline in plasma before dialysis µg per ml.	Adrenaline after 4 hours dialysis		% Adrenaline in dialysate
		in impermeate µg per ml.	in dialysate µg per ml.	
1	1.83	1.70	0.050	2.7 %
2	3.30	2.70	0.082	2.5 -
3	1 17	1.13	0 031	2.7 -
4	1 51	1.42	0.040	2.7 -
5	1 89	1.84	0.051	2.7 -
6	2 38	2 24	0.065	2.7 -
7	3.19	2.91	0 081	2.5 -
8	1 80	1.62	0.054	3.0 -
9	1.52	1.48	0.046	3.1 -
10	1.68	1.64	0.046	2.7 -

to be bound to the proteins. In tests with 10 different sera the adrenaline content of which were varying between 1.2 and 3.3 μg. per ml it was established by us that 2.5—3.1 per cent of adrenaline is dialysable, and that the adrenaline content of the im-permeate underwent a corresponding reduction. See Table IV. Thus our results explain both, — principally and quantitatively — the difference between the values obtained by determination in dialysate and by direct analysis in serum.

SUMMARY

The method of Lehmann and Michaelis for the determination of adrenaline in plasma has been tested. The original method has not come up to the requirements. By means of a Beckman spectrophotometer furnished with a fluorescence accessory set as recording instrument, and together with certain modifications of the method — inter alia — with regard to this instrument, it has been possible to carry out more accurate and objective readings and to obtain reproducible results.

Particular attention has been paid to certain important parts of the procedure as e. g. to the dependence of the fluorescence on the alkali concentrations, and on the presence of formaldehyde, the specificity of the fluorescence, and the qualities of the adrenaline used in the standard solutions. The reliability of the method has been checked by tests for recovery and by parallel analysis. Finally the question of the free dialysable adrenaline and that bound to the proteins is discussed.

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A METHOD FOR RAPID DETERMINATION OF COLLOID OSMOTIC PRESSURE

(To be concluded in the next issue)

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INTRODUCTION

In principle, the measurement of colloid osmotic pressure is very simple. However, because of practical difficulties, the direct determination of the colloid osmotic pressure of blood plasma or of serum is generally considered to be too intricate and time consuming for clinical application.

Measurements of colloid osmotic pressures are based upon the fact that certain membranes are impermeable to colloids but permeable to crystalloids. — In most arrangements for the determination of colloid osmotic pressures the fluid containing the colloids is placed inside a vessel of which a part of the wall is made up of a suitable membrane. On the outside of the membrane is placed a solution not containing colloids. When equilibrium regarding osmotic activity of the crystalloids has been established the pressure of the inside fluid is regulated until the volume remains constant.

Most osmometers intended for biological work are designed in accordance with the following considerations, cited from Krogh (1929).

"The important point is to attain equilibrium and to make sure that it has been attained. As it is usually impracticable to obtain an outside fluid which is in equilibrium beforehand, a certain amount of diffusion must take place through the membrane during the experiment. To facilitate this the membrane must be as large as possible in proportion to the volume of inside fluid; it must be as permeable as possible, i. e., just impermeable to the colloids in question and, further, the volume of outside fluid must be as small as possible, which insures in addition that the unavoidable change in composition of the inside fluid is minimized".

Among the osmometers intended for clinical application, which are designed in accordance with the above requirements, in particular an apparatus made by Krogh & Nakazava (1927), Fig. 1, has been extensively used.

In this apparatus the area of the membrane is about 2.3 cm² and the volume of inside fluid about 0.5 ml. The outside fluid is contained in a sheet of filter paper in contact with the membrane. — The time required before the readings can be made is 4 to 6 hours.

There is no doubt, however, that if a simpler arrangement to assemble and to

handle were available, the range of clinical measurements of colloid osmotic pressure could be greatly extended.

It is the aim of the present paper to introduce a fairly simple procedure for rapid and accurate measurement of the colloid osmotic pressure allowing a few simultaneous measurements to be carried out in about one hour.

MAIN FEATURES OF THE PRESENT METHOD

The method in question incorporates the following characteristic features.

1. The osmometer is made of glass and in one piece.

2. The membrane is produced and at the same time attached to the osmometer simply by dipping it in an appropriate collodion solution.

3. By the addition to the collodion solution of ethylene glycol the influence of the length of the drying period upon pore size of the membrane has been minimized.

4. Agitation of the inner fluid is carried out by means of a magnetic stirrer.

5. The correction for the capillary rise of the fluid in the measuring capillary tube is reduced by means of octyl alcohol to such an extent that the individual variations in the capillary rise of different samples of serum may be neglected.

6. During the measurements the osmometers are suspended on an arm mounted on a reading microscope, in such a way that four or six determinations can be carried out simultaneously. The level of the menisci are read on the eyepiece micrometer of the same microscope simply by rotating it on its vertical axis.
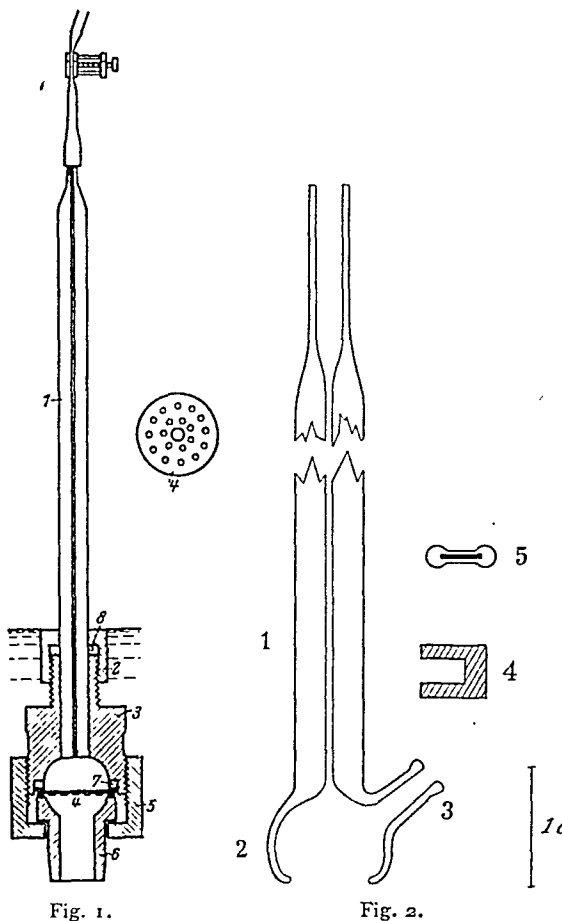
Fig. 1.   Fig. 2.

Fig. 1. Osmometer for clinical use. After Krogh & Nakazava.

Fig. 2. The writer's osmometer. 1. Measuring capillary tube, length about 18 cm, internal diameter about 0.07 cm. 2. Bulb carrying an aperture for the membrane in its bottom. Diameter of the aperture ten times the internal diameter of the capillary tube. 3. Side tube for filling the osmometer. 4. Rubber cap for closing the side tube. 5. Magnetic stirrer.

TECHNICAL DETAILS

The arrangement to be described below comprises:

1. Osmometers.

2. A devise for agitating the fluid inside the osmometers.
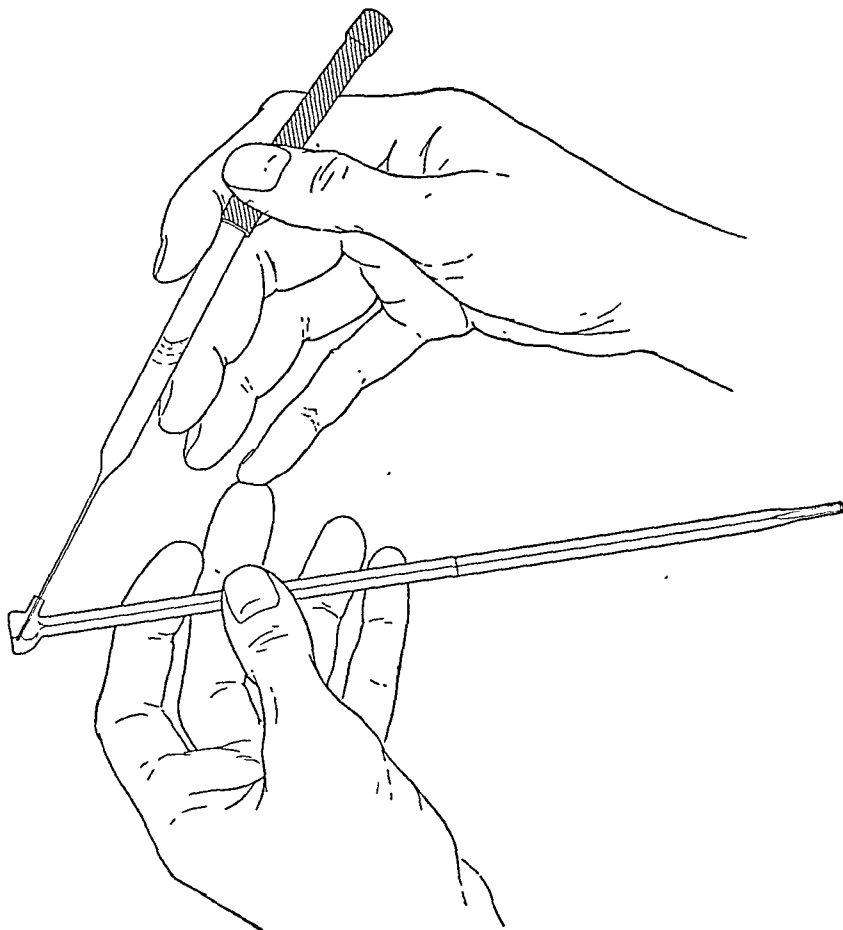
Fig. 3. Filling the osmometer. Note that the tip of the fifth finger of the right hand rests on the tip of one of the fingers of the left hand which holds the osmometer.

3. Moist chambers for the osmometers.

4. A reading microscope provided with an arm for suspending the osmometers during the measurements.

5. A constant temperature water bath.

The osmometer is made of glass, Fig. 2. It consists of a capillary glass tube (1), length about 18 cm, internal diameter about 0.07 cm, ending in a glass bulb (2), volume about 0.35 ml, with an aperture for the membrane in its base. The diameter of this aperture is ten times the diameter of the capillary, i. e., about 0.7 cm. The bulb is further provided with a short side tube (3) for filling the osmometer, internal diameter about 0.15 cm. The side tube can be closed by means

of a small rubber cap. Fig. 2, (4).

The membrane is cast and at the same time attached to the rim of the aperture by dipping the bulb in a suitable solution of collodion. Further details will be given below. The area of the membrane is about 0.38 cm².

The adverse influence of the less favorable ratio between membrane diameter and inside volume of this osmometer as compared with the osmometer of Krogh & Nakazava ($\frac{0.38}{0.35} = 1.1$ as against $\frac{2.3}{0.5} = 4.6$) is overcompensated by agitation of the fluid inside the osmometer. Before casting the membrane a small piece of iron wire, 0.05×0.4 cm enclosed in a short glass

tube closed at both ends, Fig. 2 (5), is introduced into the bulb of the osmometer where it is provisionally attached to the wall at the inlet of the side tube by means of a droplet of collodion. Later it can easily be released from this position by pushing the tip of the filling pipette through the side tube. Stirring is obtained by placing the apparatus in a suitable periodic magnetic field.

By this arrangement the time required for the crystalloid osmotic activity to attain equilibrium is greatly reduced, as it is now depending on the rate of diffusion (of water and crystalloids) across the membrane and through the outside fluid only.

The periodic magnetic field for four or six simultaneous measurements is arranged by means of an electromagnet, permanent magnets, and a blocking oscillator, Fig. 7, for feeding the electromagnet with intermittent current.[1] Technical data are given in the text of the figure.

The osmometer is filled with inner fluid as shown in Fig. 3. The outside of the pipette should be dry and no drop of fluid should adhere to the tip of the pipette, when it is introduced into the osmometer bulb. Care should be taken that air bubbles are not introduced. Even a single air bubble present in the capillary tube of the osmometer may seriously impair the measuring accuracy. If air bubbles are formed inside the bulb of the osmometer they may be removed by means of octyl alcohol. The tip of the filling pipette is wiped off with a piece of filter paper moistened with octyl alcohol. reintroduced into the bulb, and the bubbles will immediately disappear. — If the inside fluid should be unwilling to enter the measuring capillary tube, even when the osmometer is tilted so that the bulb is pointing upwards, gentle suction with the mouth may be applied at the end of the capillary tube. The capillary should be filled to at least about 15 cm above the level of the membrane.

The outside of the bulb ought not to be contaminated with inner fluid during the filling procedure.

When the inner fluid has been placed in the osmometer and the side tube closed with the

[1] The possibility of using a blocking oscillator for this purpose was pointed out to the writer by Mr. I. C. Madsen, M. Sc. who kindly indicated the diagram represented in Fig. 7.
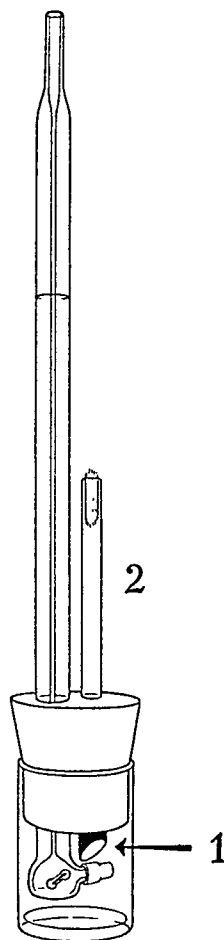
Fig. 4. Osmometer with the moist chamber. 1. A small cylindrical permanent magnet placed in a hole in the rubber stopper. 2. Glass tube stoppered with a wad of cotton wool for providing atmospheric pressure in the moist chamber.

rubber cap, the outside of the glass bulb and the membrane is thoroughly washed with physiological saline by means of a washing flask. The glass bulb is wiped off with a piece of dry filter paper, and the membrane is furnished with fresh outer fluid; a small disk of filter paper, diameter about 0.8 cm. is dipped in physiological saline and together with the adhering small drop of fluid placed in contact with the membrane. Now
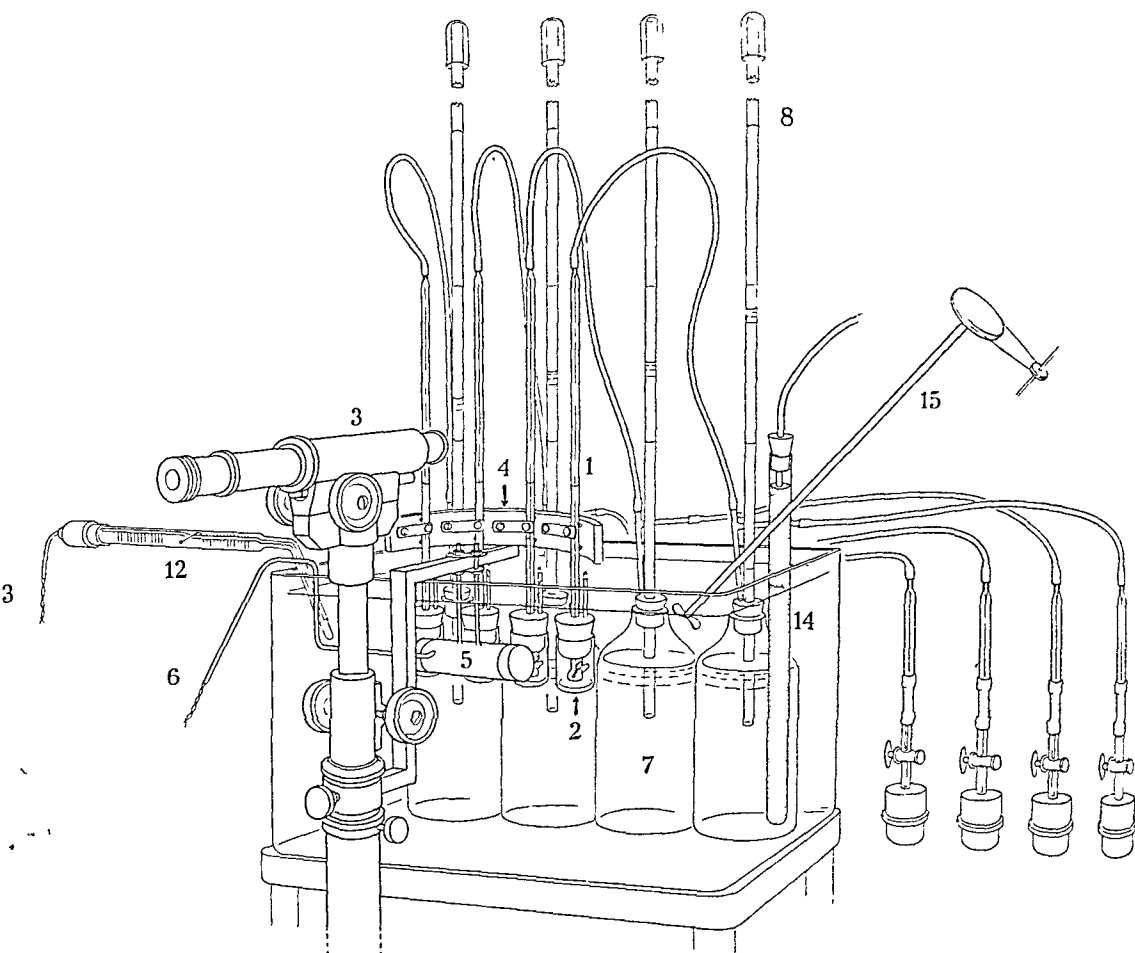
Fig. 5. The writer's arrangement for measurement of colloid osmotic pressures.   1. Capillary measuring tube of osmometer.   2. Moist chamber surrounding the bulb of osmometer.   3. Reading microscope, "Leitz"; magnification 20 diameters; working distance, 5 cm. The eyepiece is provided with a micrometer for reading the movements of the minisci of the osmometer fluids.   4. Support arm mounted on the reading microscope.   The arm is provided with clamps for holding four osmometers at the same distance from the vertical axis of the microscope.   5. Electromagnet, 500 ohms, inclosed in water tight metal tube. 6. Cable to blocking oscillator. (See Fig. 7). 7. Pressure bottle.   8. Manometer tube.   9. Pump.   10. Stopcock.   11. Narrow capillary glass tube for providing air resistance   12 Adjustable electric thermoregulator.   13. Cable to electronic relay. (See Fig. 8).   14. Heater. 50 watt.   (Compare Fig. 8).   15. Stirrer for the water bath.
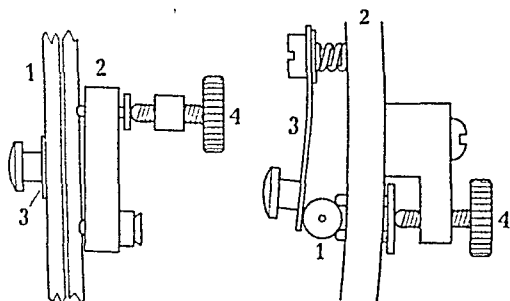
Fig. 6. Arrangement for fine focusing of minisci of osmometer capillary tube. 1. Capillary tube of osmometer. 2. Section through the arm of the osmometer support. 3. Clamp holding the capillary tube. 4. Screw for fine adjustment.

Fig. 8. Relay for controlling temperature of water bath. V. EL2. T. Terminals for cable to thermoregulator. $R_1 = 10 \times 10^6$ ohms. $R_2 = 5 \times 10^6$ ohms. $R_3 = 0.2 \times 10^6$ ohms. $R_4 = 5 \times 10^3$ ohms. $R_5 = 30 \times 10^3$ ohms. $R_6 = 30 \times 10^3$ ohms. $R_7 = 0.1 \times 10^3$ ohms. $C_1 = 0.2$ μF. P. Plunger relay. H. Terminals for cable to heater.

Fig. 7. Blocking oscillator for feeding the electromagnet (Fig. 5 (5)) with intermittent current. V. Lexington 35L6GT. L_1 and L_2 are the primary and secondary windings of an ordinary audio-frequency transformer, e. g., "Lübcke L_1-110", turn ratio 1 : 5. L_3. Electromagnet for agitating the stirrers in the osmometers. $C_1 = 1$ μF. $C_2 = 4$ μF. 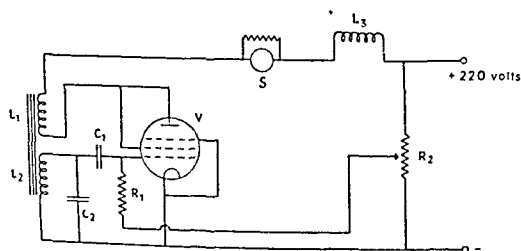$R_1 = 2 \times 10^6$ ohms. R_2. Potentiometer, 0.2×10^6 ohms. S. Flash lamp bulb, 0.04 amps, 6 volts, shunted with a suitable resistance (about 125 ohms) to prevent overloading of the lamp. The condensors applied in this apparatus should possess very high insulating properties. By means of the potentiometer the repetition frequency of the impulses can be regulated between about 15 and 50 per minute. Higher frequences can be obtained by making R_1 (or C_1) smaller. The duration of each single impulse is controlled by L_2 and C_2.

the bulb and the lower part of the osmometer is immediately enclosed in a moist chamber consisting of a flat bottomed thin glass vessel, 2.2 × 3.5 cm, and a rubber stopper, Fig. 4. The glass vessel contains just enough physiological saline solution to cover the bottom. The stopper has three holes, one for the capillary tube of the osmometer, another where the permanent magnet (1) is placed, and a third which is furnished with a short glass tube (2), about 5 cm in length, loosely stoppered with a wad of cotton wool, to provide atmospheric pressure outside the membrane.

The magnet must be strong enough to hold one end of the stirrer attached to the adjacent part of the wall of the glass bulb so that the membrane shall not be rubbed by the movements of the stirrer.

The osmometer is now suspended on an arm mounted on a reading microscope, Fig. 5, and immersed in a constant temperature water bath, kept at 25° C ± 0.01°, to at least one cm above the upper level of the stopper.

The arm holding the osmometers is provided with clamps for four or six osmometers which can be read successively on the eyepiece micrometer of the microscope simply by rotating the microscope on its vertical axis.

When several measurements are to be carried out at the same time the osmometers must be arranged so that their menisci lie at the same horizontal level. If necessary fluid may be removed from the capillary tubes by means of a narrow pipette. Fine focusing adjustments are provided as shown in Fig. 6.

A small quantity of a surface tension lowering substance, octyl alcohol, is placed in the capillary tube. A horsehair wetted with octyl alcohol (methylhexyl carbinol) is introduced through the upper end of the capillary tube dipped a few centimeters below the level of the fluid and removed.

Now the upper end of the capillary tube is connected to the pressure apparatus, Fig. 5 (7, 8, 9, 10 and 11); each osmometer having its own pressure bottle immersed in the water bath; each bottle is furnished with a manometer tube graduated from 0 to 60 cm (8). The manometer fluid is water to which is added a small quantity of methylene blue.

If the pump should not provide sufficient leak for air to escape when the stopcock, Fig. 5 (10) is opened a pinhole should be made in the rubber tube connecting (9) and (10).

The capillary tube (11) is intended to prevent sudden pressure changes in the pressure bottle; the time required for the height of the manometer fluid to be halved when the stopcock is opened should be about one minute.

Technique and Practical Problems

PREFACE

The purpose of this section of the journal is to provide space for papers dealing with problems of direct importance for the practical work of the clinical laboratories.

It will include descriptions of method and technical detail, preferably advanced beyond the experimental stage, also of new apparatus. Problems arising out of the practical interpretation of results from various investigations may also be dealt with. Finally, questions may advantageously be discussed here connected with laboratory organization, and the construction of laboratory buildings as well as the training of laboratory personnel.

A SIMPLE, TIME-SAVING ARRANGEMENT FOR THE EXACT DILUTION OF BLOOD SAMPLES FOR CELL COUNTS AND HEMOGLOBIN DETERMINATIONS

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In those Scandinavian hospitals, where a great number of blood cell counts and hemoglobin determinations have to be carried out, the Ellerman system of mixing the blood with the diluting solutions is extensively used. In this system the blood is mixed with the fluid not in mixing pipettes ("melangeurs") but in small test tubes about 12×60 mm. The tubes are set up in special racks and the amount of the diluting fluid given for the different methods is blown into the tubes, usually by means of automatic pipettes graduated for this purpose. One micropipette is enough when the blood samples are taken if this pipette is graduated in an appropriate way, for instance to 10, 20 and

25 mm³. Usually when the red cells' are to be counted 10 mm³ blood is blown into 1990 mm³ Hayems solution, for white cells 25 mm³ into 475 mm³ Türks solution and for hemoglobin 20 mm³ into 1980 mm³ 0.1 norm HCl. Of course other proportions and solutions may be used in the same system.

The system is very time-saving when greater numbers of blood tests have to be counted and it also diminishes the experimental errors. Even when this system is used, the blood counts take a considerable part of the technician's time. A certain part of the time is taken up in providing the test tubes (the "Ellerman" tubes) with
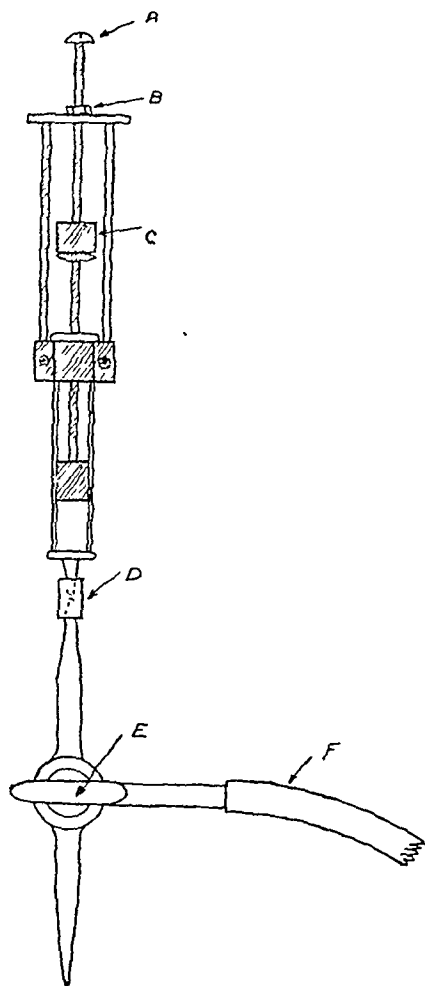
Fig. 1. Apparatus for measuring off exact
amounts of diluting fluids. Detail.

metal frame with a long screw (A) described by Krogh (1935). The position of the screw is such that it meets the hand of the plunger and thus regulates the capacity of the syringe. This arrangement has been well known for many years and used in several laboratories and clinics for other purposes. By changing the position of the screw (A) the capacity can be very carefully corrected to for instance 1990 (RC), 475 (WC) or 1980 (Hgb) mm³. This ca be gravimetrically controlled wih an error of less than 0.1 mg. The screw is locked in the desired position by a nut (B). T the open end of the syringe a three-way glass stop-cock (E) is connected by mean of a short rubber tube (D). This branc of the stop-cock has to be in direct contac wih the tip of the syringe in order to preven the tubing to influence the volume on account of its elasticity. One of the ways o the stop-cock is drawn out to a tip througl which the solution has to run out into the Ellerman-tube. By means of rubber-tubing (F) the third way of the stop-cock is connected with a storage bottle containing the diluting solution.

As the glass-plunger has to fit very closely into the syringe the friction usually will be too high to let it fall down spontaneously by its weight thus pressing the solution out. Therefore a weight, for instance one or two nuts, has to be fastened on top of the handle of the plunger to make this heavy enough to sink down by its weight. This being arranged, the capacity is corrected by means of the screw A.

The syringe is fastened in a support in an erect position with the screw (A) upwards and the free tip of the stop-cock downwards. But the fastening in the support has to be carried out in such a way that the syringe (including the stop-cock) can easily be turned upside down if air bubbles appear in it. We use a support which can be rotated on its stem. In this way it is easy to remove bubbles which often form in the solutions on standing.

the respective exact amounts of diluting solutions into which subsequently the samples have to be blown.

In order to shorten the time necessary for this measuring off of the diluting fluids a simple apparatus has been constructed. It has been in use in this laboratory for about a year and has turned out to be time-saving, exact and easy to handle (fig. 1).

It consists of an all-glass 2 ml "tuberculin"-syringe (fig. 1) provided with a

Fig 2   Arrangement of three apparatuses

The tubing between the three-way stop-cock and the storage bottle has to be long enough not to prevent this rotation of the syringe

Automatic pipettes earlier described are usually constructed to be filled by suction. But suction very often causes air-bubbles to be formed   One of the advantages of the construction described here is that there is less risque for this error to occur as the syringe is filled by pressure

The storage bottle is closed by a two-hole rubber stopper and connected to a pump by means of which the air-pressure above the solution can be raised to between 100 and 300 mm Hg.   The pressure then can press the solution to rise through a glass tube leading from the bottom of the bottle through the rubber tubing connected with the third way (F) of the stop-cock and up into the syringe, lifting its plunger until it is stopped by the screw (A). We have used a rubber bulb and manometer from a usual blood-pressure aneroid sphygmomanometer as pump and pressure-reading instrument for the three bottles in common, as illustrated in fig. 2.

When the apparatus is to be used, the suitable pressure is pumped up into the storage bottle   The stop-cock is opened so that the solution enters the syringe, lifting the plunger to the "roof" which is indicated

by a slight clicking sound. Then the stop-cock is urned so that it connects the syringe with its outlet branch. Now the weight of the plunger presses the solution out into the Ellerman-tube held below the stop-cock. The whole operation takes only a few seconds.

According to our experience there are only two errors to count with. One is the occurrence of air-bubbles in the syringe. They are easy to observe and to remove by turning the syringe upside-down. The other possible error may occur if the technician works too fast and by carelesness does not let the plunger reach the "roof" or the "floor" when filling and emptying the syringe. An ordinary technician can easily provide a rack with 60 Ellermann tubes with the proper amounts of solutions in 7 minutes. This time includes the pumping up of the air pressure in 3 bottles, removing of air-bubbles and moving the rack between the 3 syringes (for red count, white count and hemoglobin respectively). The corresponding time when the usual automatic pipettes are used is 15 min. The

corresponding time for the filling of 60 mixing pipettes ("melangeurs") with diluting solutions only is about 25 min.

The standard deviation $\left(\sqrt{\dfrac{\varepsilon\,\theta^2}{n}}\right)$ of 20 gravimetrical determinations (with water) for each of three syringes was

Table I. *Results in Gram of Graviometric Control of "Krogh" syringes.*

Gram	RC	WC	Hgb
Average content..	1.9867	0.4773	1.9795
Standard deviation	0.0051	0.0023	0.0033
Highest	1.9980	0.4790	1.9829
Lowest	1.9798	0.4734	1.9758

After continuous use for a year no change could be found in the volume of the syringes.

REFERENCE

Krogh, A.: "Syringe pipette". Ind. eng. Chem. Anal. Ed. 7, 1935, p. 130.

A QUANTITATIVE ONE-STAGE METHOD FOR THE ASSAY OF PROTHROMBIN

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INTRODUCTION

For many years there has been a controversy between the one-stage method of Quick (1935), and the two-stage method of Warner, Brinkhous & Smith (1936) for the estimation of prothrombin. The discussion has mostly concerned the validity of the underlying principles. In the various one-stage technics, oxalated (or citrated) plasma (or whole blood) is mixed with thromboplastin and calcium, and the clotting time determined. These tests consequently measure the time needed for the thrombin titer to reach the clotting level in the presence of a fixed amount of thromboplastin and calcium. Quick has insisted that the clotting time under these conditions depends entirely on the amount of prothrombin present and consequently may be used as a measure of prothrombin concentration.

The discovery of the fifth clotting factor or factor V (Owren, 1943) offered a complication in this method. It was shown that the conversion of prothrombin to thrombin needs the presence of factor V, and that the rate of this conversion depends on the amount of factor V present. The "prothrombin time" of Quick is not therefore a measure of prothrombin concentration, but varies with the absolute as well as relative concentrations of both prothrombin and factor V in the plasma. Calculations

of percentage of prothrombin using this method are erroneous. Decreased amount of factor V also gives a prolonged Quick's "prothrombin time". Furthermore, Quick's method is insensitive. Significant prolongation of the clotting time by this method does not occur until the concentration of prothrombin or factor V has dropped below 50 % of the normal. Increased concentrations of prothrombin or factor V can not be detected.

In the two-stage methods, thromboplastin and calcium are added to dilutions of defibrinated oxalated (or citrated) plasma, and the maximum amount of thrombin formed is determined by the clotting activity on fibrinogen. When factor V is reduced, the conversion of prothrombin to thrombin proceeds slowly. During the time needed for the maximal thrombin titer to occur, a part of the thrombin formed is inactivated by the antithrombin, and consequently the thrombin amount recorded will be too small. By absence of factor V no prothrombin is detected by this method. A modification of the two-stage method eliminating the errors caused by variations in factor V concentrations is described previously (Owren 1947, page 260).

In the following paper a one-stage method for the estimation of prothrombin is presented. It represents a modification of a previously described method (Owren 1947,

page 265), and gives an exact quantitative estimation of prothrombin regardless and uninfluenced by the amount of factor V present.

PRINCIPLE

In a clotting mixture all coagulation factors are kept constant except prothrombin. The clotting time of this mixture will then be entirely dependent on the amount of prothrombin present.

REAGENTS

Prothrombin-free Ox Plasma.

Ox blood (9 vol.) is mixed with 1 vol. 2.5 % (w/v) potassium oxalate monohydrate solution and the plasma obtained by centrifugation. Prothrombin is removed by using asbestos-paper filter pads containing 30 % asbestos. Asbestos differs from other prothrombin adsorbents ($Mg(OH)_2$, $Al(OH)_3$ and $Ca_3(PO_4)_2$) because it adsorbs prothrombin selectively, leaving factor V in plasma. The following procedure is recommended: The plasma is stored over night at 0° C and centrifuged to remove fatty material which tends to clog the filters. The ~~plasma is then passed through a clarifying ~~ter (containing about 20 % asbestos) and ~~ then twice through pads containing 30 % ~~asbestos. (Seitz E. K. or Hodgkinson, Wookey Hole, Sommerset, England). The plasma is neutralized to pH 7.3 by addition of N/2 HCl. It is stored at or below —20° C in small bottles or glass-tubes stopped with corks, in quantities suitable for one day's use.

Thromboplastin.

It is of considerable practical advantage to use a thromboplastin-extract with a constant and reproducable activity. This is not achieved by extraction of dried brain substance, not even by a standardized extraction technic. The following method is recommended:

Human brain is freed from pia and blood vessels, washed, and the gray substance is ground in a mortar with 0.9 % NaCl-

solution to a fine emulsion. The emul~~sion~~ fication is facilitated by applying a mec~~hanical~~ nical blender (for instance "Ato-Mi~~x~~ blender and disintegrator). The emulsi~~on~~ is diluted with 0.9 % NaCl-solution, ~~to~~ which 20 % veronal buffer is added. ~~A~~ total of approximately 5 ml solution p~~er~~ gram brain substance is used for the e~~x~~traction. The extract is left standing ~~a~~ couple of hours at 45° C, after which it ~~is~~ strongly centrifuged for 15 minutes. T~~he~~ precipitate is discarded, and the gray~~ish~~ colloidal solution is frozen at —20° C ~~in~~ portions corresponding to the daily ~~re~~quirement. Before being used it is thaw~~ed~~ at 37° C. This extract gives a Quick "prothrombin time" in normal plasma ~~of~~ approximately 15 seconds. When stor~~ed~~ frozen at a temperature below —20° ~~C~~ the extract remains unchanged duri~~ng~~ several months.

Veronal buffer. To 570 ml 0.10 ~~M~~ sodium diethylbarbiturate add 430 ~~ml~~ n/10 HCl and 5.67 g NaCl. Dilute with ~~an~~ equal volume 0.9 % NaCl-solution.

Calcium chloride solution. The optim~~al~~ calcium concentration for recalcification ~~is~~ determined experimentally for the plasma which is used. It is approximate~~ly~~ 0.030 M $CaCl_2$, varying slightly with t~~he~~ oxalate concentration of the ox plasm~~a~~ which again depends on the hematocrit val~~ue~~ of the applied ox blood.

PRACTICAL PROCEDURE

The blood sample is collected with o~~ne~~ part 2 % (w/v) potassium oxalate mono~~hydrate solution to 9 parts blood. Aft~~er~~ centrifugation a dilution of 1 part oxala~~te~~ plasma and 9 parts veronal buffer is ma~~de~~ (0.2 ml plasma + 1.8 ml buffer).

0.20 ml prothrombin-free ox plasma ~~is~~ mixed in a small test-tube together wi~~th~~ 0.20 ml plasma dilution and 0.20 ml throm~~bo~~boplastin solution. The mixture is place~~d~~ in a water-bath at 37° C for 5 minut~~es~~ after which 0.20 ml calcium chloride sol~~u~~tion is added for optimal recalcificatio~~n~~

and the clotting time is determined by stop watch in the usual manner.

The clotting time in this system depends entirely on the prothrombin content of the plasma dilution. The quantitative determination requires a correlation graph which shows the relation between the clotting time in this system and the prothrombin content of the plasma dilution. This correlation graph is made by applying different dilutions of a normal plasma. The 10 % dilution is made in the usual manner. Higher dilutions are made by a veronal buffer containing potassium oxalate 30 mg per 100 ml solution for the maintenance of the oxalate concentration in the different dilutions. The correlation graph with the dilutions given in per cent as abscissa, and the clotting time in seconds as ordinate, becomes a straight line on double logarithmic paper.

The standard plasma used for determination of the correlation graph should be frozen in small portions in the same way as the other reagents, so that the stability of the system may be controlled at any time. The prothrombin remains unchanged during a long period of time when deep frozen.

By the examination of a sufficient number of normal plasmas, the normal graph is determined.

COMMENTARY

The concentration of factor V in ox plasma is 3—4 times higher than in human plasma. The application of prothrombin-free ox plasma therefore causes a constant surplus of factor V in the coagulation mixture. The variations of factor V in the plasma dilutions which are to be examined are so small that they are without influence. The fibrinogen concentration in the ox plasma is approximately 0.40 %. The fibrinogen concentration of the mixture is consequently optimal (0.10 %) at all times, and practically speaking constant.

The oxalate concentration of the mixture is primarily determined by the oxalate content of the ox plasma, and is therefore relatively constant, regardless of minor variations of the oxalate concentration in the plasmas which are to be examined. The coagulation will therefore always take place at optimal calcium concentration. (In Quick's method the oxalate concentration of the plasma varies with the hematocrit value of the blood used, consequently the recalcification does not always become optimal).

This method has proved to be far better suited for the control of the dicoumarol treatment than Quick's method.

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PROBLEMS REGARDING THE COUNTII
OF RED BLOOD CELLS

(RESULTS OF INVESTIGATIONS)

BY H. F. LANGE AND H. PALMER

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During research, the authors detected considerable uncertainty in the technique for the determination of the erythrocyte concentration of the blood. They found it therefore necessary to make this technique subject to a detailed examination, in order to enable the registration of the methodological errors in their widest sense. Each individual step of the proceedure, and all conditions which might possibly influence the result, were examined more closely.

For these investigations, Buerker's counting chambers were used, and separate pipets: 10 cmm's mercury-calibrated pipets for blood, and 2-ml full-pipets for the dilution liquid.

A series of six venous punctures in the course of 10 minutes in the same three individuals, showed that the erythrocyte concentration in the venous blood was constant by the technique applied, and with this value as a standard, comparative examination was carried out in capillary blood.

The factors which might possibly influence the result of the erythrocyte counts, were divided into three categories:

1. *Purely technical errors,* which comprise, among other things, apparatus and reagents.
2. *Technical-physiological errors,* including the technique employed in collecting the blood samples, and the reaction of the organism against the executed operation.
3. *Purely physiological variations.* This group does not concern detection of errors in its proper sense, but an examination of the factors which influence the concentration of blood corpuscles in the organism.

All factors within these three categories were studied. The paper mainly deals with the factors which were found to influence the results, so that the work is a criticism of the weak points in the determination.

In the following, a brief account of these "weak points", their nature, magnitude, and how they may be counteracted, will be given.

1. Technical Errors.

The Counting Chambers: Physical measurements of the height of three Buerker counting chambers showed errors of $+8$, $+4$, and $+3.5\%$ respectively. (These are average figures, as the height was different in different places within each counting chamber). Physical calibration of the counting chambers at intervals, is therefore recommended, as calibration with the aid of blood cell counting was found to give unreliable results.

By comparison of the counting chambers *with and without clamps,* the latter were found to give the most reliable values, as, among other things, it was found to be easier without these clamps to obtain Newton's colour rings of the same order during the fixing of the cover glass against the cross beams.

The actual *filling of the counting chambers* gave no rise to special variations, and superfluous liquid could be removed immediately, without any interference with the result.

The distribution of the blood cells within the counting chamber showed a systematical unequal distribution, the inner part of the field (nearest to the central beam) having from 4.7 to 16.5 % higher erythrocyte concentration than the outer part. It is therefore recommended that the same squares should always be used during counting, preferably along a diagonal line.

Rocking of the mixing glass: Mixing during one minute, prior to the liquid being transferred into the counting chamber, was found to give sufficient security.

Dilution liquid: A 0.9 % NaCl-solution gave equally reliable results as Hayem's solution, but the NaCl concentration should be accurate, and the solution must not be too old. The results remained unchanged when the blood dilution liquid mixture was left standing some time between the collecting of the blood sample, and the counting.

2. *Technical-physiological Errors.*

"Hyperemisation" of the finger by massaging with ether-soaked cotton wool gave reduced values on an average of 5 %. A light washing is therefore recommended.

The use of a scarificator gave greater reflex capillary changes, (and thereby increased erythrocyte concentrations) than the use of a thin, sharp knife.

When the blood was *pressed out,* the values were on an average 7.5 (up to 26) % too high. An incision, 3—4 mm long, and 4 mm deep produced sufficient bleeding.

The wiping off of the first drop of blood gave undesirable variations. The blood should be allowed to flow freely before the sample is collected.

Ear or finger blood? The erythrocyte values in finger blood were found to be both the most estable and the nearest to venous blood. Finger blood should therefore be taken in preference. The finger blood which flowed 20—50 seconds after the incision, was found to give the most

constant results, and may be regarded as nearest to the "normal". As a practical rule it is recommended to draw up the blood into the pipet half a minute after the incision has been made.

3. *Physiological Variations.*

These were mainly "relative", i. e., the erythrocyte concentration was found to vary inversely with the blood volume. (When, for instance, liquid + crystalloids leave the blood vessels, colloids and blood corpuscles will be retained, and their concentration will be relatively greater).

These changes in the blood concentration may be *rapid* or *slow*. The *rapid* changes are caused by intake of fluids, work, and changes in the body position. The *slow* changes are caused by changes in the time of the year, changes in the altitude, pregnancy and age.

Of the *rapid* changes, intake of fluid plays no practical role, while considerable activity and changes in the body position may bring about such great changes in the blood concentration (under special conditions up to 20 %), that it is necessary to make considerable consideration for these factors when looking for normal "basal" values, and to standardise the conditions of the experiment. Food intake, on the other hand, does not appear to be of any importance and there is no need for the patients to be "fasting" at the time when the blood samples are collected.

Among the *slow* changes, only the well known variations during pregnancy, and during the newborn period, as well as the infant period, are of any practical importance

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A SUITABLE ABSORBENT FOR OXYGEN IN GAS ANALYSIS

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According to Haldane's method for gas analysis and to subsequent modifications, the oxygen content of a gas sample is absorbed by potassium pyrogallate or alkaline hydrosulfite solution. Stone & Beeson (1936) and Stone & Skavinski (1945) have described in the technical literature an acetic acid solution of chromous chloride which in some respects is superior to the others. The solution has been used by us for some time for determining oxygen in samples of expired air, and has been prepared in the following way.

150 grams of granulous zinc, 20—30 mesh, are immersed in 50 ml 3 M hydrochloric acid for 30 seconds. 50 ml 0.013 M ... uric chloride solution are added and ... mixture stirred rapidly for about 3 ... minutes. After decanting, the amalgam is washed a couple of times with dest. water.

Chromic chloride crystals are dissolved without heating in 2 M acetic acid to make about 100 ml 2 M chromic chloride solution. This solution is added to the amalgam in an Erlenmeyer flask which is sealed by a rubber stopper and stirred for about 5 minutes. The solution is then deep blue in colour and can be transferred to the absorption chamber of the gas analysis apparatus which is sealed with oil as usual.

This solution absorbs oxygen quicker than the alkaline pyrogallate or hydrosulfite solutions. When using the latter with sodium anthrahydroquinone-β-sulfonate, we had to wash 35 times to attain complete absorption whereas only 8 washings were required with the new solution. It has a capacity of about 11 volumes of oxygen per volume of reagent, and is effective as long as there is chromous ions to react with the oxygen. When the solution becomes inactive the colour turns from blue to green. The other solutions have a higher capacity, but the rate of absorption decreases rapidly with decreasing oxygen pressure in the gas sample. In consequence the oxygen absorbing power of the pyrogallate and hydrosulfite solutions cannot be fully used. We have found that the acetic acid solution of chromous chloride can be used 3 to 4 times longer than the alkaline hydrosulfite solution.

As the solution is acid in reaction, carbon dioxide need not be eliminated before the determination of oxygen is made. Hydrogen gas analysis should not be made in apparatuses containing this solution.

SUMMARY

Attention is drawn to an oxygen absorbing acetic acid solution of chromous chloride described in the technical literature. This solution has been used in analyses of expired air according to Haldane and has proved to absorbe oxygen at 3—4 times the rate of alkaline hydrosulfite solution.

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A METHOD FOR RAPID DETERMINATION OF COLLOID OSMOTIC PRESSURE

(Continued from page 76)

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THE MEMBRANES

The possibility of measuring colloid osmotic pressures with the osmometer in question is entirely dependent upon the possibility of producing suitable membranes as outlined above.

Membranes for the present osmometer should fulfil the following requirements:

1. The membranes should combine the greatest possible permeability to crystalloids with absolute impermeability to proteins, or, in other words, the maximum pore diameter should be as large as is safely compatible with impermeability to proteins and the average pore diameter should be as near the maximum pore diameter as possible.

2. Uniform membranes should be produced without difficulty. Variations in the length of the drying period should not influence too critically the pore diameter, neither should the normal range of temperature and humidity of the air in the laboratory seriously influence the permeability of the membranes.

3. The membranes must be strong enough to withstand a hydrostatic pressure of up to about 50 cm of water pressure without being stretched beyond their elastic limit.

4. The membranes must adhere firmly to the glass rim of the aperture and must be able to withstand a maximum pressure of the inner fluid of at least 50 cm of water pressure without allowing even minute amounts of fluid to intrude between the glass bulb and the adhering part of the membrane.

It is beyond the scope of this paper to report in detail the numerous experiments carried out to establish which composition of the collodion solution and which procedure for casting the membranes should be preferred for the present purpose. A single procedure is described in the following, which has turned out to yield membranes which fully satisfy the above requirements.

The grade of collodion used was "Collodion Gurr" produced by Edward Gurr, Ltd., 108 Waterford Road, London S.W. 6.

"Collodion Gurr" is a preparation of nitrated cotton wool moistened with a varying quantity of ethyl alcohol and generally containing a small amount of water also.

A small quantity of this preparation is thoroughly dried, and a collodion solution of the following composition prepared:

"Collodion Gurr", dried .... gram 4
Ethylene glycol .... ml   5.5
Ethyl alcohol, absolute .... ml 125
Ether, anesthetic .... ml 80

This solution will keep for months in a well stoppered glass bottle (cork stopper).

Before use a small quantity of collodion solution is centrifuged in a tube, closed with a cork stopper, and the clear fluid transferred to a clean vessel in which the dippings are made.

The permeability of collodion membranes produced from the above solution will be determined mainly by the amount of gelifying substances, ethyl alcohol and ethylene glycol, or precipitants as water, which are present in the membranes when they are soaked with water after the end of the drying process. The presence of even small amounts of water during the gelifying process, i.e., during the evaporation of the ether, will seriously impair the quality of the membranes by making the pores less uniform and the membranes more fragile.

The addition of ethylene glycol, a non-volatile gelifyer, to solutions of collodion intended for preparation of membranes, was introduced by Pierce (1927) who obtained membranes of reproducible permeability by drying them for 24 hours in a specially designed apparatus containing $CaCl_2$. By changing the ratio collodion glycol he was able to grade the permeability of his membranes.

It is not feasible to simplify the procedure of Pierce by drying the membranes in the air of the laboratory. Ethylene glycol is very hygroscopic, and even small changes of temperature, humidity, and circulation of the air will considerably alter the properties of the membranes.

However, it was found, what for theoretical reasons should be anticipated, that the electric refrigerator of the laboratory, which was kept at 1° C, would provide ideal environmental conditions for drying the membranes. Membranes pro-

duced from the above solution would posses practically the same permeability whether they are dried in the refrigerator overnight or left there for several days. Because of the low humidity and the low temperature in the refrigerator the amount of fluid left in the membranes after the evaporation of ether and alcohol will be very constant and keep practically constant for a long time owing to the very slow rate of evaporation of ethylene glycol at 1° C.

The only difficulty encountered when using membranes produced from collodion solutions containing glycol has been that the membranes will not always adhere firmly enough to the glass bulb to satisfy requirement no. 4 page 87.

This drawback was overcome by pretreating the osmometers as follows:

Before the osmometers are used for the first time the outside of the bulbs from the rim of the aperture and about 0.3 cm upwards are frosted. A rubber stopper is placed in the aperture and the glass etched with a paste containing hydrogen fluoride. Before casting the membrane a thin layer of a 20 per cent solution of shellac in ethyl alcohol is applied to the frosted area. After drying for a few minutes at room temperature the osmometer is placed in an oven at 150° for at least 30 minutes. The layer of shellac covering the frosted area will now adhere firmly to the glass, and the collodion film formed, when the membrane is cast, will stick to the shellac sheet with sufficient strength even when the osmometer is soaked with water for days or weeks.

The collodion membranes are made as follows:

The osmometer bulbs are dipped four times in the collodion solution. For the first two dippings the surface of the collodion solution should only just be touched, whereas, for the last two dippings the aperture of the bulb should be submerged 0.3 to 0.4 cm into the fluid whereafter the osmometer should be immediately removed.

The first dipping will leave a film of collodion solution covering the aperture. After the dipping, the osmometer is held in a nearly vertical position (angle with the vertical about 10°) and rotated slowly round the axis of the capillary tube for a few seconds, whereafter the osmometer is suspended in vertical position with the membrane downwards. Fig. 9 (1).
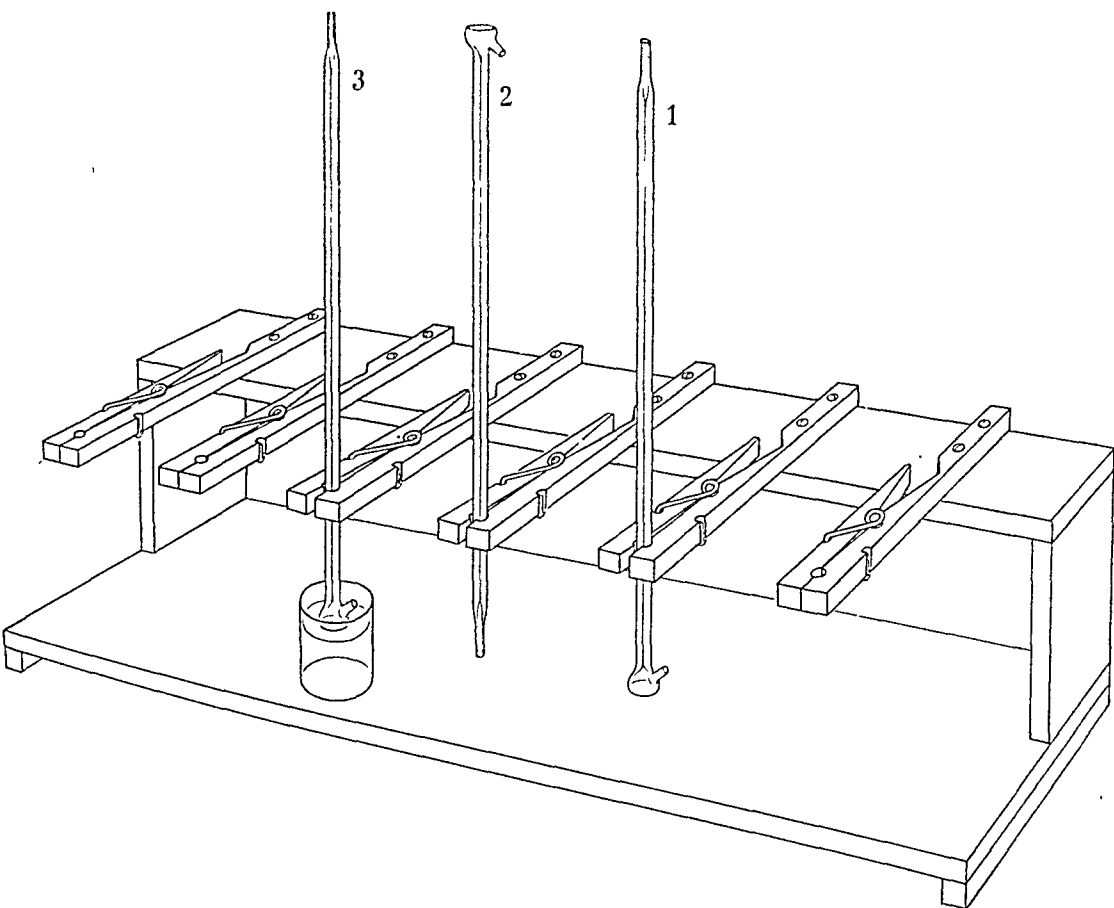
Fig. 9. *Support with test tube clamps for holding osmometers.* The osmometers are stored with water-filled bulbs and arranged so that the membranes are touching a water surface (3).

At ordinary room temperature the membrane formed by evaporation of ether (and alcohol) from the film will be strong enough to stand the second dipping after about 2 minutes. After the second dipping the osmometer is treated as after the first dipping, with the only difference that it should now be rotated for 1.5 to 2 minutes, until the adhering drop of collodion solution has been uniformly spread over the whole of the membrane and the gelifying processes have begun, before it is suspended.

The interval between the second and the third dipping should be at least 10 minutes to make sure that the membrane will be strong enough to stand the pressure of the fluid during the third dipping.

After the third dipping, in which the membrane is dipped 0.3 to 0.4 cm below the surface of the collodion solution and immediately removed, the osmometer is held for a few seconds in an oblique position, with the capillary tube forming an angle of about 45° with the vertical, membrane downwards, side tube pointing upwards, to allow a single drop of the collodion solution to escape from the bulb; whereafter the osmometer is again rotated for 1.5 to 2 minutes, angle with the vertical 30° to 45°, and suspended as after the second dipping for at least 10 minutes.

The fourth and final dipping is carried out exactly as the third dipping and a single drop of collodion is again allowed to drip from the bulb, but thereafter the osmometer is rotated in horizontal position for 20 to 30 seconds, whereafter the bulb is further elevated until the axis forms an angle with the vertical of about 45°, membrane pointing upwards. After 2 to 3 minutes the osmo-

meter is again suspended in the support, but now with the membrane pointing upwards, Fig. 9 (2).

The casting of the membrane is now finished. The osmometer may at once be transferred to the refrigerator, or if 'the air of the laboratory is reasonably dry the osmometer may for the sake of convenience be allowed to stay in the laboratory for up to 1 hour.

When the membranes have been dried for at least 12 hours in the refrigerator they may be removed. The osmometers are at once dipped into distilled water, whereafter they should be stored with the bulbs filled with water and membranes just touching a water surface, Fig. 9(3).

After the further lapse of a few hours the membranes may be used for measurements.

The thickness of membranes produced in this way should be about 0.005 cm.

The membranes' permeability to water will depend on the ratio, collodion/glycol, and may be graded simply by changing the concentration of glycol in the collodion solution.

Until sufficient experience has been gained regarding the influence on the properties of the membranes of the environmental conditions prevailing in the laboratory and in the refrigerator, the first measurement to be carried out on new membranes should be the determination of the membranes' permeability to water.

The permeability of a collodion membrane to water may conveniently be expressed by the so called "minute number", which is by definition the time required for the filtration of one cm^3 of water through 1 cm^2 of membrane at a pressure difference across the membrane of 1 atm.

Good membranes, with uniform pore size, should possess absolute impermeability to proteins when the minute number exceeds 60 to 70.

Under the conditions prevailing in this laboratory membranes, produced as described above, will possess minute numbers of 80 to 90 and they have always turned out to be perfectly protein tight.

If, however, membranes are produced in a room where the humidity of the air is excessive, or where the temperature is so low that humidity of the expired air of the operator will condense on the membranes, which in the initial stages of drying are further cooled by the evaporation of ether, it may be advisable to place the membranes at a distance of about 30 cm from a 60 watt incandescent lamp during the initial drying processes which take place in the laboratory.

Practical details regarding the determination of the minute number of the membranes together with a description of the test for protein tigthness will be given in the following chapters.

MEASUREMENTS

Measurements carried out with the present arrangement are all based upon readings of the heights of the columns of fluid in the osmometer capillary tube and in the manometer tube of the pressure bottle, together with simultaneous readings on the eyepiece micrometer of the microscope of the movements of the meniscus of the fluid in the osmometer tube.

From these readings the hydrostatic pressure difference between the two sides of the membrane and the rate of filtration of fluid through the membrane can be calculated.

The hydrostatic pressure difference, p, between the fluids just inside and just outside the membrane is given by

$$p = h_1 \times d_1 - e_1 + h_2 \times d_2 \pm e_x \quad (I)$$

where h_1 and h_2 denote the heights (cm), and d_1 and d_2 the densities of the fluids in the osmometer and manometer tube respectively; e_1 is the capillary rise of the octyl alcohol covered surface of the inner fluid in the osmometer capillary tube; $\pm e_x$ represents the unknown effect of the surface tension of the outer fluid.

Carrying out measurements on serum, density about 1.025, with the osmometer filled up to 15 to 17 cm above the level of the membrane, we may for the sake of convenience, substitute $h_1 \times d_1$ by $h_1 + 0.4$ cm; furthermore, we will ignore e_x, which probably will only involve the introduction of a minor essentially systematic error. Based on measurements of the capillary rise of different sera covered with octyl alcohol, methylhexyl carbinol, we may take e_1 equal to $\frac{0.055}{r}$ (for water it is $\frac{0.073}{r}$), where r is the radius of the capillary tube (cm). Thus we have

$$p = h_1 + 0.4 - \frac{0.055}{r} + h_2 \qquad (II)$$

For the osmometers in question with capillary diameter 0.07 cm we get

$$p = h_1 - 1.2 + h_2 \qquad (III)$$

It was found convenient to place a mark on the osmometer tube at a distance of $10 + \left(\frac{0.055}{r} - 0.4\right)$ cm from the level of the membrane and carry out the measurement from this mark by means of a small millimeter rule.

For calculating the minute number, M, of a membrane we must know the linear velocity, v_m (cm/min.), of water passing the membrane under a definite pressure, p cm of water.

Substituting 1 atm. by 1000 cm of water pressure we have

$$M = 10^{-3} \times \frac{p}{v_m} \qquad (IV)$$

or, replacing v_m by $v_c \frac{r^2}{R^2}$, where v_c denotes the velocity (cm/min.) of the meniscus in the capillary tube and R and r are the radii of membrane aperture and capillary tube of the osmometer, we get

$$M = 10^{-3} \times \frac{p}{v_c} \times \frac{R^2}{r^2} \qquad (V)$$

The meniscus is watched through a microscope provided with an eyepiece micrometer with 100 divisions. The length of the tube of the microscope is adjusted so that one division of the micrometer corresponds to exactly 0.005 cm in the object viewed, and we may substitute v_c by $0.005 \times v_d$, where v_d is the velocity of the meniscus expressed in divisions/min.

So we have

$$M = 0.2 \times \frac{p}{v_d} \times \frac{R^2}{r^2} \qquad (VI)$$

If $\frac{R}{r}$ is exactly $= 10$ we get

$$M = 20 \frac{p}{v_d} \qquad (VII)$$

which equations may be applied for the determination of the minute number.

DETERMINATION OF THE MINUTE NUMBER OF THE MEMBRANES

The osmometer is filled with distilled water, furnished with distilled water as outer fluid, placed in the moist chamber the bottom of which is covered with distilled water also, and placed in the water bath as described above. After 30 minutes temperature equilibrium will be established and v_d is determined at a pressure of 50 cm. The

minute number is now calculated from the equation

$$M = \frac{1000}{v_d}. \qquad (VIII)$$

If $\frac{R}{r}$ is not exactly 10, M should be figured out from

$$M = \frac{10\,R^2}{v_d\,r^2}. \qquad (IX)$$

DETERMINATION OF THE COLLOID OSMOTIC PRESSURE OF BLOOD SERUM

Before introducing the sample of serum to be measured, the osmometer should be washed with physiological saline; the capillary tube is connected to a water jet pump and the bulb intermittently dipped in physiological saline. Now the membrane is provisionally furnished with outer fluid contained in a small disk of filter paper, and the inside of the bulb is washed with a small quantity of the sample of serum to be measured. The washing serum which is indtroduced by means of the filling pipette may advantageously be removed by suction through the side tube, but care must be taken that the pressure inside the bulb is not reduced more than about 50 cm (of water) below atmospheric pressure as, otherwise, the membrane may be damaged. A suitable gentle suction may be obtained if the rubber tube connecting the suction pump and the side tube of the osmometer is so wide that no suction is obtained unless the rubber tube is gently pressed against the osmometer bulb. One washing with serum is sufficient.

Now the osmometer is filled with serum and placed in the water bath as described page 73.

A pressure of 40 cm is immediately applied, agitation started, and the osmometer left for 30 minutes when temperature equilibrium will be established and measurements can begin.

If the volume of outer fluid is not excessive, only a small drop should adhere to the disk of filter paper carrying the outer fluid, and, if the minute number of the membrane does not exceed about 120, equilibrium regarding the osmotic activity of the crystalloids will practically be attained at the same time.

The pressure is now regulated until the meniscus in the measuring capillary tube remains at a constant level. The readings of the osmotic pressure can be finished about one hour after the osmometer has been placed in the water bath.

It should be ascertained that the meniscus will begin to rise or fall if the hydrostatic pressure is lowered or elevated by 0.2 to 0.3 cm.

For membranes with minute numbers about 100 a difference of 0.25 cm between the equilibrium pressure and the pressure applied should cause the meniscus to move about 0.05 divisions per minute.

Quadruple measurements on the same sample of blood serum have been made several times. Readings made after one hour will agree with a maximum deviation from the mean of $\pm$ 0.5 cm of water pressure; which deviation, if the measurements were extended to a duration of two hours would decrease to $\pm$ 0.3 cm. For blood serum the difference between readings obtained after one hour and the final constant readings reached after 1.5 to 2 hours very seldom exceeded 0.4 cm of water pressure.

TEST FOR PROTEINTIGHTNESS

Until sufficient experience has been gained regarding the influence of the environmental conditions during the drying of the membranes, new membranes should always be tested for proteintightness. For membranes having minute numbers below 70 the test is indispensable.

The test for proteintightness is conveniently carried out in continuation of the first measurement of osmotic pressure undertaken with the membrane. The disk of filter paper carrying the outer fluid is removed. The osmometer is again placed in its moist chamber and a total pressure of 60 cm applied. After a few hours a small drop of ultrafiltrate may be collected by means of a very narrow pipette. The drop of ultrafiltrate is delivered into a small quantity, about 0.05 ml of "Spiegler's reagent"[1] previously placed on a micro slide. The slide is now viewed with the naked eye against a dark background. The protein concentration of serum diluted up to 500 times still gives a visible precipitation.

CLEANING OSMOMETERS

After use the osmometers must be thoroughly washed. The rubber cap is removed, the upper end of the capillary tube connected to a water jet pump and the bulb intermittently dipped, first in physiological saline, and thereafter in distilled water.

The osmometers are stored with bulbs filled with distilled water and membranes submerged 0.1 to 0.2 cm below a water surface.

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[1] Spiegler's reagent consists of mercuric bichloride g 4, tartaric acid g 2, glycerol g 10, and distilled water g 100.

Owing to the short duration of each measurement it is not necessary to use disinfectants, which will often spoil the membranes. Growth of microorganisms in collodion membranes may be prevented by storing the membranes in a 50 per cent solution of glucose, which procedure will not influence the properties of the membranes (Hammarsten 1948).

When membranes have been used for measurements for about a fortnight, they generally have to be discarded. Probably because of growth of microorganisms the membranes will become fragile and their minute number will rise.

The membrane is removed by means of a knife and the layer of collodion and shellac adhering to the bulb is carefully scraped off. The osmometer is thoroughly washed, the side tube of the osmometer being connected to the water jet pump, and the "upper" end of the capillary tube intermittently dipped into distilled water. After drying, the osmometer bulb is furnished with a fresh coating of shellac and new membranes made as described above.

If the rule is strictly followed, that osmometers containing biological fluids should always be washed with saline before they come into contact with tap water or distilled water, it will only be necessary to clean them with dicromate-sulfuric acid or the like very occasionally.

ERRORS

On account of restricted space the errors with which the present method might be encumbered cannot be dealt with in detail in this paper.

The weakest point of the method is undoubtedly the rubber caps applied for closing

the side tubes of the osmometers. Because of the strange combination of elasticity and viscosity which is characteristic of most plastics slow volume changes which may proceed for hours will take place after the caps have been placed on the side tubes. Also volume changes caused by the uptake of water in the rubber caps have to be considered.

Volume changes corresponding to a movement (rise as well as fall) of the meniscus of up to 0.1 division per minute have been noticed even 60 minutes after the caps have been placed on the side tube of a "model osmometer" with no aperture for membrane.

Another cause of error to be taken into consideration is the influence of variations of the volume of outer fluid. If the disk of filter paper carrying the outer fluid does not contain at least sufficient fluid to make its surface look shiny, the capillary suction power of the outer fluid will markedly influence the reproducibility of the measurements.

COMMENTS

Even if the procedure described in this paper is intended first of all for clinical use, there can be no doubt that it can also advantageously be used in experimental work.

In experimental physiology a method for the determination of the colloid osmotic pressure of the very small samples of blood serum which can be secured from small laboratory animals is urgently needed.

Experiments to elucidate if the present method can be adapted for this purpose are in progress.

SUMMARY

The present paper describes a new and fairly simple osmometer arrangement suitable for clinical measurement of the colloid osmotic pressure of blood serum.

The simultaneous measurements of the colloid osmotic pressures of four samples of serum can be carried out in one hour. Quadruple measurements on the same serum will agree with a maximum deviation from the mean of $\pm$ 0.5 cm of water pressure.

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ACQUIRED HEMOLYTIC ANAEMIA AND LYMPHOBLASTOMA

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Investigations of later years have thrown much light upon the pathogenesis of the various types of hemolytic anaemias (Loutit & Mollison, Dameshek & Miller, Dameshek & Schwartz).

Yet many questions remain unsolved. In animals, one may produce a hemolytic anaemia very similar to the spontaneous hemolytic anaemias in man, by injections of antiserum against the species (Dameshek and Schwartz 1938). This does not explain all cases of hemolytic anaemia occuring in various diseases, e.g. in lymphogranuloma, lymphosarcoma, giant cell tumors (Rosenthal and Wassermann 1947) or in ovarian cysts, leucaemia and hepatic diseases (Singer and Dameshek 1945).

Some of these cases of acquired hemolytic anaemia, however, have been shown to be due to an anomaly in the serum, to the existence of an antibody against the red cells.

It has repeatedly been shown (Loutit and Mollison, 1943, Dameshek and Schwartz, 1938, Owren, 1947) that the mechanism of destruction of the red cells in these conditions is quite different from that seen in the congenital hemolytic jaundice. In the acquired type the destruction his the patients own red cells as well as the red cells transfused from a normal healthy donor

(Dacie and Mollison 1943). The detailed mechanism of destruction is unknown. Dameshek originally explained the destruction as hemolysis. Recent investigations point to a more complicated mechanism (Dameshek and Miller, 1943, Kass, 1945, Loutit and Mollison, 1943).

The diagnosis of this type of hemolytic anaemia is often facilitated by Coombs' reaction with antihuman globulin rabbit serum (Boorman, Dodd, and Loutit, 1946). A positive reaction indicates a sensitization of the red cells. In congenital hemolytic anaemia, Coombs' reaction is always negative.

Recent investigations point to the spleen as a possible factor in the sensitization process. It is considered to be an important organ for antibody production, and splenectomy has in some cases been followed by reduced hemolysis. In other cases this has failed to influence hemolysis, showing that the spleen is not the only organ responsible. Acquired hemolytic anaemia occasionally occurs in conditions pointing to the possibility of other organs as antibody producers, e.g. in a case of a dermoid cyst of the ovary, with promptly cure following removal of the cyst (Singer and Dameshek, 1945).

In the following report a case of acquired hemolytic anaemia is described, caused by

circulating antibody against the red cells. Experiments are performed which indicates that the production of antibody may have taken place outside the spleen, in a lymphoblastoma.

CASE REPORT

E. M., engineer's wife, born in 1912 in Berlin, was admitted to the medical department on the 31th of May 1948.

2 children, no abortions, Pregnancies and deliveries without complication. She had never received blood transfusion.

In 1943 a left-side nephrectomy for pyonephrosis.

Family history was without any known incidence of anaemia.

Her present disease started in May 1948 with weakness, slight fever, increased sedimentation rate (78—100 mm) and aching pains in the back.

On admission she was pale with slight jaundice. Temperture was 37.5° C. In the left hypochondrium a deeply seated tumour was felt, the size of a grape-fruit. Otherwise negative findings. Spleen and liver were not palpable, nor were the peripheral lymph nodes enlarged.

Laboratory examinations: Hb. 42 %, (Haldane) R. B. C. 1.9 millions, W. B. C. 6600, icterus index 6 (Meulengracht). Blood platelets 400 000, sedimentation rate 125 mm (1 hour). Differential count showed a moderate shift to the left. Serum albumin 4.2 per cent, serum globulin 2.1 per cent. Wassermann negative. No blood in the faeces. The urine had a dark yellow colour. Schlesinger's test for urobilin was strongly positive, otherwise urine examination was negative.

The erythrocytes in blood smears showed a striking tendency to agglutinate in dense masses.

The sternal marrow showed hyperactive erythropoiesis. 3 7 per cent reticulocytes were found in the peripheral blood. The osmotic fragility of the erythrocytes was slightly diminished (0.34—0.22 per cent).

During the first days the haemoglobin and erythrocyte values showed a slight increasing tendency, but the following days the values decreased again, the haemoglobin to 43 per cent and red corpuscles to 2 millions, and transfusion treatment was started.

Clinical course

As the weeks passed, the tumour in the lef hypochondrium increased in size and at last presented itself as a hard nodular, slightly tender tumour of the size of a newborn child's head. I did not move on respiration and was assumed to be retroperitoneally seated. X-ray treatment on the tumour was given for 12 days without significant improvement of the blood values.

Blood transfusions also had only transient effect and were at last discontinued.

Following each transfusion but one, a massive haematuria appeared. Fresh urine contained masses of erythrocytes as well as free haemoglobin. This may be explained by assuming multiple emboli of agglutinated erythrocytes to be present in the kidneys.

The patient died on July 30th 1948.

Autopsy

About 400 ml yellowish fluid was found in each pleural cavity. Normal lungs and heart.

A yeollowish-white soft bronchial gland, 3 cm long was found.

Spleen: Weight 475 g. Surface smooth, consistency somewhat hard. An infarction of the size of a walnut was found.

Liver: Weight 1730 g. Structure normal.

Several mesenteric glands were slightly enlarged, to the size of a bean, most of them soft, pale. Behind the abdominal aorta a mass of similar soft, pale glands, the size of a walnut, was found. Apparently this mass represented the remnants of the great abdominal tumour.

Microscopically all glands showed the same picture with masses of lymphoid cells with very scarce cytoplasm, divided by fibrous septa. The nuclei were mostly large and round, deeply stained, with distinct chromatin network. Many atypical mitoses. Neither fibrils nor stellate cells were seen. — A section stained by silver nitrate showed no fibrils.

The normal lymphoid structure of the spleen was largely replaced by the same great atypical cells.

No tumour cells were found on microscopical examination of the liver, the kidneys or the heart.

Diagnosis: Lymphoblastoma.
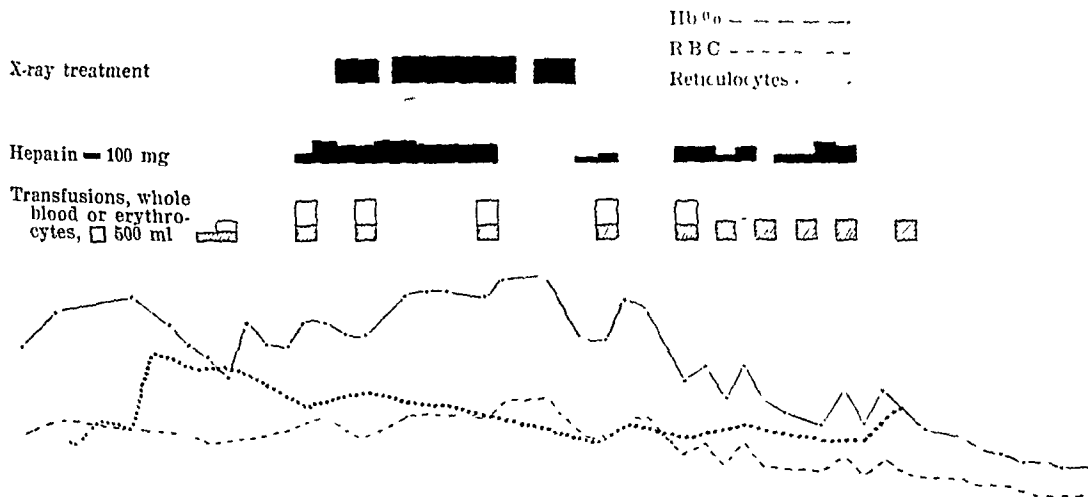
SEROLOGICAL EXAMINATION

A sterile blood sample which was drawn before the condition had been diagnosed and irradiation instituted, was completely coagulated and showed no signs of hemolysis after one hour at room temperature. Agglutinated cells could be found in the serum after shaking of the clot at room temperature. The agglutination was more marked at $+5°$ C. The patient's serum containing agglutinated cells was incubated at $+5°$ C for 15 minutes in order to obtain maximal agglutination, and then at $37°$ C for 15 minutes during which period considerable haemolysis occurred (Donath-Landsteiner's phenomenon). Clinical ABO typing of the cells was not feasible owing to the spontaneous agglutination which was highly accentuated by addition of test serums (reaction as AB), or by addition of AB serum.

The cells were washed four times with about 50 volumes of saline at $37°$ C and the blood type then determined as O M R_1R_2. Similar washing at room temperature still gave an AB result, although less marked than before. Coombs' test was only slightly positive. ABO type and Rh type (Rh positive or Rh negative) were also checked by absorption tests using the patients unwashed cells.

The isoagglutinin titers in serum after absorption of the cold agglutinins with half a volume of 0 cells at $+5°$ C were as follows:

Titer against A cells: 512.
»   »   B cells: 128.
»   »   0 cells: 0.

These values are within the normal limit. Cold agglutinin titers in nonabsorbed serum against 0 cells were as follows:

$5°$ C	$20°$ C	$37°$ C
4096	256	8

In order to determine the amount and quality of the agglutinins linked to the patient's cells the sample was incubated overnight at $+5°$ C. The next morning cells were taken from the coagulum and washed rapidly once with 50 volumes of ice-cooled physiological saline in order to remove rem-

nants of serum. These cells were then treated as follows:

One volume of cells (0.1 ml) was added to 50 volumes of sterile physiological saline, repeatedly shaken for ten minutes and then centrifugated. The fluid was then decanted and the washing repeated ten times. The washing (splitting-off of agglutinins) was carried out parallely at 37°, 20°, and 5° C with physiological saline of the same temperature. All washing fluids were then titrated against an 1 per cent suspension of 0 cells at 37°, 20°, and 5° C. When the patient died, the body was placed overnight in a refrigerator (freezing temperature). Autopsy specimens were taken the next day and placed in saline. Pieces weighing 0.1 g were removed from

the central portions of these specimens (tumour, liver, spleen, kidney) and squeezed between two slides and the tissue and tissue fluid transferred to centrifuge tubes by means of 10 ml of ice-cooled physiological saline under sterile conditions. The contents were immediately centrifuged at high speed and the washing fluid discarded. These washed tissues were then treated in exactly the same manner as described for the erythrocytes above (10 ml physiological saline were used for each washing). The various washing fluids were then titrated as described above. The titers of the six first washing fluids from erythrocytes and tumour tissue are shown below.

T a b l e I. *Splitting-off of agglutinins.*

Titer values in successive washing fluids from red cells and tumour tissue, titrated at 3 different temperatures against a 1 per cent suspension of 0 cells in physiological saline.

Titration temperature:	5° C		20° C		37° C	
Washing fluids from:	Cells	Tumor	Cells	Tumor	Cells	Tumor
Splitting-off temperature: 37° C			Titer values			
Washing fluid number: 1	32	32	32	32	2	4
» » » 2	16	16	16	16	0	2
» » » 3	8	8	8	8	0	4
» » » 4	2	4	2	4	0	2
» » » 5	2	8	0	0	0	0
» » » 6	2	4	0	2	0	0
Splitting-off temperature: 20° C						
Washing fluid number: 1	32	16	32	8	0	0
» » » 2	16	16	16	8	0	2
» » » 3	8	4	8	4	0	0
» » » 4	8	2	4	2	0	0
» » » 5	4	4	4	0	0	0
» » » 6	4	4	4	0	0	0
Splitting-off temperature: 5° C						
Washing fluid number: 1	2	8	2	4	0	0
» » » 2	2	8	2	2	0	0
» » » 3	2	2	2	4	0	0
» » » 4	2	2	2	2	0	0
» » » 5	2	2	2	0	0	0
» » » 6	2	2	2	0	0	0

No agglutinins were derived from erythrocytes or tumour tissue by further washing at 37° C, but traces of agglutinins were present in the fluids from the seventh and eighth washing performed at room temperature and even after the tenth washing at + 5° C (traces indicate a titer of 2 at + 5° C).

Similar washing of liver and spleen specimens (0.1 g of tissue + 10 ml physiological saline) gave no agglutinins. Renal tissue, however, gave traces (titer 2) in the first two washing fluids at 37°, 20°, and 5° C.

The agglutinins derived from erythrocytes and tumour tissue withstood incubation at 56° for 30 minutes without any demonstrable reduction in titer value, but were inactivated when heated to 70° C for 5 minutes. They were easily absorbable by any human red cells at lower temperatures (+ 5° C), and agglutinated all bloods independently of ABO, MN, and Rh type with the same intensity, by the same temperature when tested against a total of 96 blood samples, partly at room temperature and partly at 37° C. Erythrocytes from guinea-pigs and rabbits were not agglutinated by the derived agglutinins. The atypical agglutinins in serum were considerable more thermostable. They agglutinated all 0 blood samples (40) with the same intensity.

DISCUSSION

Progressive anaemia, without any haemorrhages, increased erythropoiesis and the serological findings, characterise the anaemia as haemolytic, caused by an autoagglutinin.

Aquired haemolytic anaemia has repeatedly been observed as a symptomatic con-dition in other disorders, such as malignant lymphogranuloma, lymphosarcoma, colloid cancer of the stomach, giant cell tumour, and leucaemia (Rosenthal & Wassermann 1947), dermoid cyst of the ovary (cured by removal of the cyst), hepatitis and infectious diseases (Singer & Dameshek 1945). It must be stressed, however, that apart from one case among those described by Singer & Dameshek (1945), none of the patients had auto-agglutinins in their serum.

In this patient we had reason to assume that a connection existed between the haematological disorder and the steadily increasing abdominal tumour.

The natural plan for the treatment according to this theory seemed to be the following: If the tumour could be removed, either surgically or by x-ray treatment, the source of the haemolytic agents should thereby be eliminated or reduced. As the nature of the tumour favoured the diagnosis of a lymphoma, x-ray treatment was started.

As shown in the table 1, the blood values at first showed increasing tendency during the x-ray treatment. What made us still more optimistic, was that the tumour during the treatment decreased rapidly in size. After 10 days treatment, the tumour was not palpable any longer. But at the same time the palpation of the abdominal organs was facilitated due to the disappearance of the tumour, and revealed an enlarged spleen, and the liver was felt 2 inches below the right costal margin.

The graph shows the rapidly decreasing blood values after the finishing of the x-ray treatment. That this anaemia is not due to the x-ray damage of the bone marrow, is shown by the reticulocyte values.

We consider it justified to believe that the rapidly increasing anaemia during this period is due to an augmentation of the same process which caused the patients anaemia before the x-ray treatment, in other words, to an increase in the destruction of the red cells.

The investigations seem to favour the assumption that the lymphoblastoma was responsible for the content and the production of the agglutinins in this case.

Investigations of the antibody formation in lymph nodes by Ehrich & Harris (1945) have given valuable contribution to this question. Later, Harris & al. in a series of experiments have not only shown that a strong antibody formation takes place in lymphoid tissue when exposed to an antigen, but their results seem to give very strong evidence that this antibody formation is taking place inside the lymphoid cells themselves.

Cases of lymphatic leucaemia with haemolytic phases have been recorded (Bøe, 1946).

The behaviour of the atypical agglutinins in this case at various temperatures justifies the term "cold agglutinins". Somewhat contradictory to the assumption that the tumour is the agglutinin producer is the very close parallelism between tumour and erythrocytes in the splitting-off experiment. On the other hand the very small output of agglutinins from other organs favour the idea of the tumour as the agglutinin producer. The reason for the small content of agglutinins in renal tissue and particularly in liver and spleen tissue is unkown.

A 20 per cent physiological saline extract of a four days old liver specimen showed traces of agglutinins, which, however, disappeared completely after incubation for 30 minutes at 37° C. A mixture of equal volumes of liver extract and agglutinins derived from erythrocytes showed a decreased agglutinating potency following similar incubation compared to a control where physiological saline had been added instead of liver extract (enzymatic action?).

In view of these observations we think it possible that the lymphoblastoma has been the source of the agglutinin production.

Theoretically, the complete removal of this lymphoid tissue should have resulted in the cessation of the haemolytic processes. — If the lymphoid tissue is destroyed by x-ray treatment, the destruction may probably result in the release of great amounts of antibodies, and thereby in an increase of the haemolytic disorder.

The final result will depend on whether or to what degree the lymphatic tissue is removed (destroyed).

Though we have no proof for the above mentioned explanation for the clinical course in this case, there is some evidence that although the tumour was induced to disappear by x-ray treatment, this treatment has possibly after all done more harm than good because of the large amounts of agglutinin probably released. Moreover, the autopsy showed lymphoblastomic tissue elsewhere in the organism, which had escaped the x-ray influence.

SUMMARY

In a woman suffering from lymphoblastoma and acquired haemolytic anaemia, large amounts of cold agglutinins were found in her serum, and could also be splitt off from the tumour and erythrocytes. It is suggested that the tumour was the agglutinin producer.

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THE RENAL CLEARANCE OF THIOSULPHATE IN MAN

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Determination of sodium-thiosulphate-clearance as a measure of the glomerular filtration rate in humans was described by Newman, Gilman and Philips in 1946.

Studies of the partial functions of the kidney such as glomerular filtration rate have no longer an interest limited to the kidney and its diseases alone. Their scope of interest has been widened, especially in regard to the heart function and general circulation, to oedema formation in heart failure (Warren and Stead, 1944) and related problems. One can therefore say that determination of the glomerular filtration rate has an increasing *actuality,* and so has also the problem of finding a simple and exact method for this determination.

At present, the standard method is the *inulin* clearance test which was introduced in 1934 by Richards, Westfall and Bott, and in 1935 by Shannon and Smith. These and other authors have put forth evidence which points to inulin as a true glomerular substance, which is freely filtered by the glomeruli and quantitatively excreted in the urine without being influenced in any way by the tubular epithelium. As a true measure of the glomerular filtration rate its clearance is not influenced by its plasma con-

centration or by diuresis. This is now generally, although not unanimously, accepted.

The common method for determination of inulin in blood and urine is by colorimetry with diphenylamine after hydrolysis to fructose with strong mineral acid (Corcoran and Page, 1939, Alwing, Rubin and Miller, 1940.) However we find the method relatively complicated and timeconsuming, and hardly suited for the ordinary hospital laboratory, at least in our country. We have used the method quite extensively, and already at this stage we should like to state that in our hands the scatter of the results is wider for inulin clearance than for the other three clearances mentioned in this paper. To this one can add that inulin is an expensive substance and, at least at present, hard to get hold of.

Among the glomerular substances *creatinine* has long been in the foreground. Creatinine is a natural component of the blood, but its plasma concentration is so low (about 1 mg%) that determinations until relatively recently have been inaccurate.

For this reason Rehberg in his creatinine clearance method of 1926 gave creatinine per os and that way increased the plasma concentration to more than 20 mg%. The

history of this exogenous creatinine clearance lies outside the scope of the present paper.

As for determination of endogenous creatinine clearance which was introduced in 1937 by Popper and 1938 by Miller and Winkler this method has been made more simple and accurate by the modern photo-electric colorimeters.

The real hindrance to a general use of endogenous "creatinine" clearance as a measure of glomerular filtration rate is this: what one measures in the plasma with Jaffe's picrate reaction is not only creatinine but also some other chromogenous substance, which however is not excreted in the urine. With a very ingenious method Miller and Dubos (1937) have shown that of all this chromogenous matter in the plasma, creatinine constitutes 80 to 100 %. This ought to make the endogenous "creatinine" clearance a little lower than the real glomerular filtration rate, and this seems to be the case as will be shown later in this paper. We have here a method well suited for clinical work, as recently stressed by Addis (1948). A more extensive study of the endogenous "creatinine" clearance will be published from our laboratory in a short time, with a more detailed discussion of its advantages and disadvantages.

In 1940 Smith, Finkelstein and Smith stated that the electrolytes either are re-absorbed by the tubuli (Na, K, Cl, nitrate, thiocyanate, sulphate, uric acid etc.) or excreted by the tubuli (Phenol red, hippurate, diodrast etc.). They pointed out that the road further ahead lies in hunting for an electrolyte which is freely filtered in glomeruli, but not excreted or reabsorbed in tubuli. It was therefore with great interest that one heard in 1946 that Gilman,

Philips and Koelle had found that *sodium-thiosulphate* behaves in this way in dogs. In 13 experiments with 46 clearance periods they found the ratio thiosulphate clearance/exogenous creatinine clearance from 0.9 to 1.1. (In dogs exogenous creatinine clearance and inulin clearance are equal.) In other words thiosulphate seemed to be quite an unusual electrolyte. The same ratio is also found in acidotic dogs (Pitts and Lotspeich, 1947) and in rabbits (Bing and Effersøe, 1948).

The excretion of thiosulphate in relation to inulin was examined in man by Newman, Gilman and Philips (1946). After one injection of thiosulphate the clearance was determined on a falling plasma concentration curve, from 60 to about 6 mg%. They found in 15 patients, including 8 with renal disease, in altogether 73 clearance periods, that the clearance of thiosulphate largely equalled inulin clearance. The average ratio thiosulphate clearance/inulin clearance was 0.99, with a scatter between 1.25 and 0.75.

Later Betty Crawford (1948) examined 7 patients without renal disease in 39 clearance periods (at constant plasma concentration) and found an average ratio thiosulphate clearance/inulin clearance of 0.98. Simultaneously large doses of hippurate or diodrast were given intravenously. By their tubular excretion these substances block the functional capacity of the tubular epithelium. It was shown that the clearances of thiosulphate and inulin were unaffected by that procedure. Brod and Sirota (1948) examined 7 individuals without renal disease in 47 clearance periods and found the ratio endogenous "creatinine" clearance/thiosulphate clearance equal to 0.95. In 9 patients with

renal disease the ratio was not fully satis-factory, being 1.10.

According to common opinion sodium-thiosulphate has no toxic action, given in 10 % solution intravenously in a total amount of up to 150 ml. Care must be taken that the solution is correctly made. Oxyd-ation may cause formation of tetrathionate which is highly nephro-toxic.

Determination of sodium-thiosulphate is in the principle simple. It is done as an iodine titration. Thiosulphate is stable in urine and plasma filtrate for several days. The substance is cheap, and easy to procure.

It seems that we here have an electrolyte which fills the requirements for a glomerular substance, which is nontoxic and easy to determine. If all this should be sufficiently confirmed the substance would take pre-cedence over inulin and creatinine in deter-minations of the glomerular filtration rate. However, as stated by Newman et al. (1946) further investigations are needed, especially with constant infusion and accompanied by determiations of diodrast or sodiumhippurate clearance.

OWN INVESTIGATIONS

During the course of our investigations of renal blood flow and renal function in nor-mals and cardiac patients we were recently faced by the calamity that our supply of inulin was running out. Owing to inulin being hard to get, we decided to try out the clearance of sodiumthiosulphate when we still had some inulin left, in order to see if it was possible to let thiosulphate take the place of the other substance later on.

Sodiumhippurate was used simultaneously for determinations of renal blood flow.

Determinations of renal blood flow and the clearances of thiosulphate and endogenous "creatinine" have been performed in 2 nor-mals and 13 cardiac cases. In 9 of the cardiac cases inulin clearance was also done. It is a rather short series of investigations, but this is somewhat compensated by the fact that in almost all cases the determin-ations were done during 6 consecutive clear-ance periods.

METHODS

We have used the standardized technique for clearance determinations given by Homer Smith and his associates (1945).

Care has been taken to insure a most constant infusion rate, and consequently a constant plasma concentration of sodiumhippurate, inulin and sodiumthiosulphate. The bladder has always been emptied with an indwelling catheter, and the last drops secured through expression after instillation of 20 ml water + 20 ml air through the catheter. For the sake of accuracy a sufficiently high diuresis has been produced by water drinking. Most clearance periods have lasted 10 minutes. Of the 6 periods the two first periods have been resting, the next two during artificial hypoxaemia produced by respiration in 9.5 % O_2, and the last two periods have again been in rest after a few minutes 100 % O_2.

This procedure is a step in our examinations of the circulation in cardiac failure, and the results in this respect will be published elsewhere. It is of interest in the present connection while in this way fluctuations in the clearance values both in the single patient, and from case to case are secured. This gives a good foundation for the comparison of clearance values at different levels.

In the priming infusion, 50 ml of a solution of sodiumthiosulphate has been given. Lately this amount has been increased, up to 75 ml according to the patient's weight.

The sustaining infusion has been made up of 50 to 75 ml 10 % sodiumthiosulphate solution + the usual amounts of inulin and sodiumhippurate in

normal saline, altogether 300—350 ml. The infusion rate has been 80 drops per minute. (Supposed to be about 4 ml/min.) In this way the plasmaconcentration of sodiumthiosulphate has reached 10 to 20 mg%. The thiosulphate solution has been the N. Ph. 10 % solution, not more than two days old.

Toxic reactions [1] have not been observed, although one of the patients, a waiter, some hours after the experiment developed a characteristic attack of delirium tremens, which however did not last very long. Although it is not reasonable to charge the thiosulphate with this happening, it should be mentioned here.

Thiosulphate has been determined by an iodine titration worked out by Claus Brun of Copenhagen, but not yet published. C. Bruns method is a simplification of the method given by Newman et al. (1946). Satisfactory results with Bruns method were obtained by Bing and Effersøe (1948). We have not ourselves compared the two methods, but this has been done by C. Brun who in a personal comunication has confirmed that they give identical results. We have tried the method with known solutions of sodiumthiosulphate, and found satisfactory results. Double determinations have always been performed, and there has almost been no divergence.

The plasma determinations have been done after sedimentation with sodiumtungstate and sulphuric acid in a dilution 1 : 5 (1 ml plasma + 1 ml 10 % sodium tungstate + 1 ml ⅔ N sulphuric acid + 2 ml water). Centrifugation is needed in order to secure a waterclear filtrate. Urine is diluted 1 : 25 to 1 : 50. To 3 ml plasmafiltrate or diluted urine is added 1 ml 2 N hydrochloric acid and a few drops of a starch solution. Titration is then directly performed with a 1/1000 N iodine solution in a 2 ml all glass burette (no rubber tubing).

The iodine solution is titrated against a stable solution of sodiumthiosulphate about 1/400 N. The reaction follows the equation: $2Na_2S_2O_3 + J_2 \rightarrow Na_2S_4O_6 + 2NaJ$. 1/1000 iodine solution is made of 1/10 N iodine solution. Iodine solutions are unstable while J_2 evaporates. KJ is therefore added to the solution in order to bind $J_2 \cdot (J_2 + KJ = KJ_3$ b: $J_2 \div$). The iodine solution must be made of fresh destilled water, and renewed weekly.

Inulin is determined by the diphenylamine method after a modification given by Corcoran and Page (1939) and Alwing, Rubin and Miller (1939).

Creatinine is determined by the Folin Wu method with Jaffe's picrate reaction. The filtrate for sodiumthiosulphate has been used also for the creatinine determination. (Plasmadilution 1 : 5.) Colorimetration in a Klett Summerson photoelectric apparatus.

RESULTS

The plasmaconcentration of sodiumthiosulphate has averaged about 15 mg%, and in the different patients varied between 9 and 21 mg%. In the single patient the plasmaconcentration usually has been very constant, and has not varied more than 3 mg%. The plasma concentration of inulin in the single patient has also been quite constant. The diureses has usually been about 6 ml/min., but has varied considerably in each patient. The maximal variations in the single experiments have been from 4 to 13 ml/min.

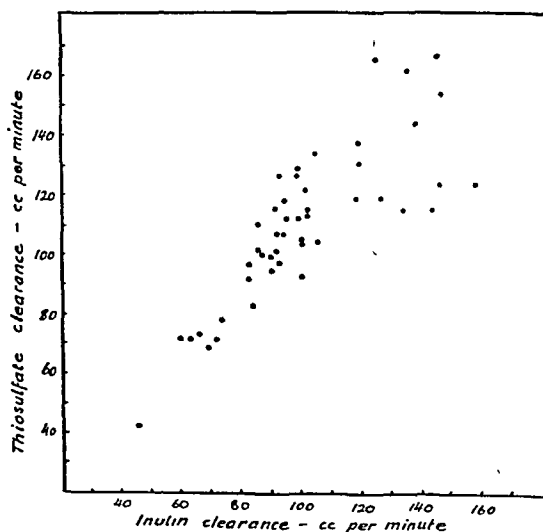All the simultaneous determinations of *inulin and thiosulphateclearance* are shown

Fig. 1.

diagramatically in Fig. 1. One will see that the dots largely are massed around a straight line. The dots represent 49 clearance periods in 9 cardiac patients without renal disease. The average value of the ratio thiosulphate clearance/inulin clearance is *1.08,* with a standard deviation of $\pm$ 0.13. The ratio is thus close to 1. In the diagram is seen that three of the low ratios have an unusually great deviation from the average. These values are all from the same patient. The three other clearance periods in this case showed the normal ratio. The explanation of the greater deviation is probably an error in the chemical analysis, but we have wanted to exclude no figure from this calculation.

ratio thiosulphate clearance/inulin clearance in every single patient, has varied between 0.88 and 1.24.

All the simultaneous determinations of the *clearances of thiosulphate and endogenous*
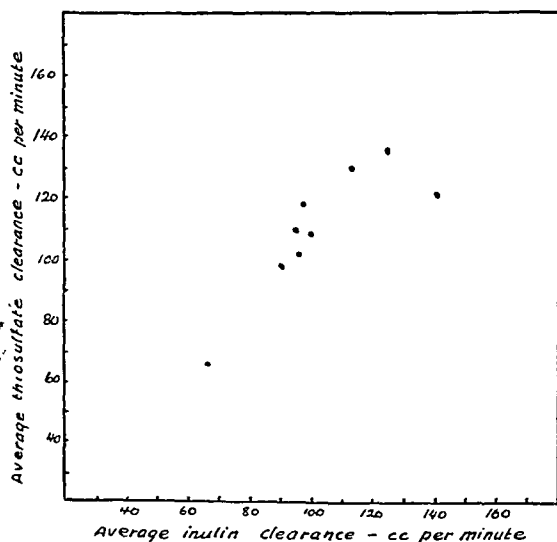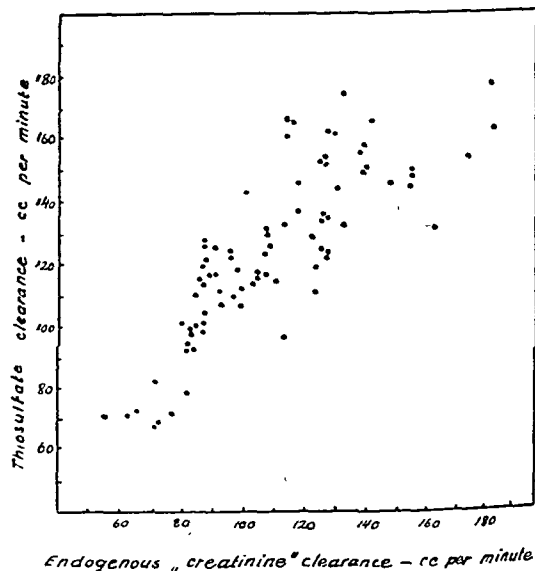

Fig. 3.

Fig. 2.

The average values of the clearance periods in each of the nine patients are recorded in a diagram (Fig. 2). It is seen that also here the dots are collected around a straight line. The only value that deviates more pronouncedly represents the patient mentioned above. The average value of the
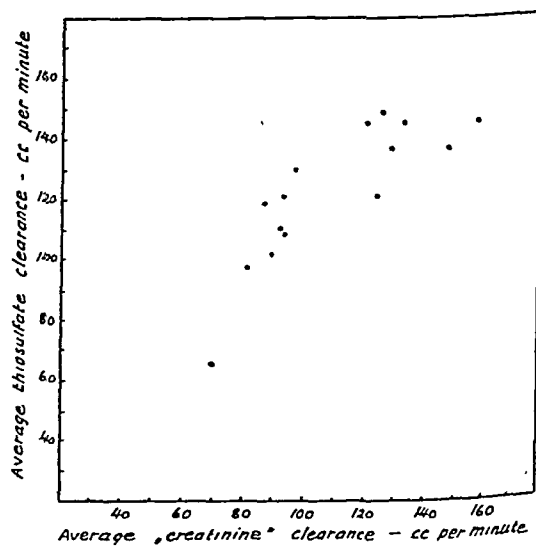

Fig. 4

"*creatinine*" are also recorded in a diagram (Fig. 3). Also here is seen that the dots largely are massed around a straight line. The diagram represents 85 clearance periods in 15 individuals. The average ratio thiosulphate clearance/endogenous "creatinine"-clearance is 1.14 with a standard deviation of ± 0.16. Analysis has shown that the deviation from 1 is statistically significant. In another diagram (Fig. 4) the averages of the clearance in each of the 15 patients are recorded. Also here the dots collect around a straight line. The average ratio thiosulphate clearance/endogenous "creatinine" clearance has varied between 0.94 and 1,37.

DISCUSSION

Mathematical analysis shows that the difference between the clearance of thiosulphate and inulin may be accidental. More experiments are needed in order to settle the question. As mentioned above other investigators have found the ratio very close to 1.0.

If the small deviation from 1 is real this could signify that a small part of the thiosulphate is secreted in the tubules.

But the deviation can also be explained by faulty methods. On the whole we can not be absolutely certain that the values for inulin are more correct than those for thiosulphate. The determination of inulin is in itself quite complicated and can give rise to errors. Thiosulphate is, as mentioned before, determined with a method which is different from what most other investigators have used, and this may be the cause of small differences in results. Finely the cautious dosage of thiosulphate with a correspondingly low plasma concentration increases the effect of errors of titration.

Our investigations have also shown a positive correlation between the clearances of thiosulphate and endogenous "creatinine". Statistically it can be said that the difference of 14 % is certain, and it is unnecessary to increase the number of determinations in order to have this confirmed.

The results are in agreement with the conception that the endogenous "creatinine" clearance is a little lower than the glomerular filtration rate. Also these simultaneous determinations of thiosulphate and endogenous "creatinine" clearance suggest that the clearance of thiosulphate must lie very close to inulin clearance and the real glomerular filtration rate.

Our results also show that the clearance of thiosulphate is independent of the diuresis.

In all these investigations the clearance of sodiumhippurate has also been determined. The concentration of hippurate in plasma has been around 2 to 3 mg%. As far as can be seen this low concentration has no influence upon the clearance of thiosulphate. Neither does hippurate in these concentrations influence the determination of thiosulphate after Bruns method, such as otherwise has been found (Elliot and Scott 1948).

CONCLUSIONS

Our investigations have shown that simultaneous determinations of thiosulphate and inulin clearance give practically equal results. This confirms the results originally made by Newman et al., and later by Crawford (1948). Simultaneous determinations of thiosulphate and endogenous "creatinine" clearance also suggest that the clearance of

thiosulphate must lie very close to inulin clearance and the true glomerular filtration rate.

Altogether it is probable that the clearance of thiosulphate equals the glomerular filtration rate in cardiac patients also, and the clearance of sodiumthiosulphate can be recommended as an accurate and simple method for the determination of the glomerular filtration rate.

SUMMARY

Simultaneous clearances of sodiumthiosulphate, inulin, endogenous "creatinine" and sodiumhippurate have been determined in two normals and 13 cardiac patients, in altogether 134 periods. The results with regard to the usefulnes of thiosulphate clearance as a measure of glomerular filtration rate is presented and discussed.

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COMPARISON OF THE FICK AND 'HAMILTON METHODS FOR THE DETERMINATION OF CARDIAC OUTPUT IN MAN[1]

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Hamilton et al. in 1931 developed a technique for the determination of the cardiac output in man and in intact animals based on a principle set forth by Hering 1842 and Stewart in 1898. They showed the validity of the formulas in simple flow models and in heart lung preparations. In 1948 Hamilton and his group in Georgia in collaboration with the Cournand group at Bellevue Hospital published a direct comparison in unanesthetized man of the dye injection method and the direct Fick method for c.o. determinations. In most cases there was a fair agreement but in single instances the two methods gave differences of more than 30 per cent. The material included normal cases at rest and during work as well as patients in different stages of heart failure.

Nylin has recently adopted a similar method using tagged red cells (1949).

It is the purpose of this paper to compare the values for cardiac output simultaneously obtained with the dye injection method and the direct Fick method in normal man and in cases with different kinds of heart disease, using a somewhat modified technique. The details of our technique are described in a following paper (Lagerlof et al. 1949). All

mixed venous blood specimens were sampled from the pulmonary artery. The dye was in all cases injected in the pulmonary artery and sampled from the brachial artery.

Material. The c.o. was determined simultaneously 69 times with the two techniques in 50 cases. Six had essentially normal circulation, 18 hypertensive cardiovascular disease, 12 mitral valvular disease. Some cases of pulmonary disease, congenital heart disease, pregnancy or arteriosclerotic heart disease were also studied. Most values were determined with the patient basal. When two pairs of determinations have been done in the same individual the second usually refers to the condition after the administration of a drug (cedilanid, theofylline or dehydroergotamine).

RESULTS

In Table I the oxygen consumption, A—V O_2-difference, and c.o. according to the direct Fick and the dye injection method are tabulated. The cases are grouped according to the stages of compensation using the criteria of the New York Heart Association.

Some data obtained by statistical treatment of our, the Bellevue and the Georgia series are given in Table II. In all series the

Table 1.   *a. Hypertension.*

Case Nr.	Group	Age sex	Weight Kg	Body Surf. Area m²	Pulse Rate	Oxygen Consumption ml./min.	A−V O₂ diff. ml./l.	Cardiac Output l./min. Fick	Cardiac Output l./min. Hamilton	Difference l./min.
140	I	47 ♂	77.3	1.95	72	256	58	4.41	4.39	0.02
143	I	50 ♀	60	1.68	81	210	42	5.0	5.35	−0.35
152	I	45 ♂	57.5	1.74	64	237	42	5.65	6.16	−0.51
»	I	45 ♂	57.5	1.74	66	287	48	5.97	5.52	0.45
163	I	26 ♀	59	1.70	92	234	35	6.68	7.08	−0.40
163	I	26 ♀	59	1.70	—	222	36	6.16	7.24	−1.08
149	I – II	48 ♂	70.1	1.84	90	364	34	10.7	10.18	0.52
149	I – II	48 ♂	70.1	1.84	69	227	44	5.16	6.68	−1.52
181	I – II	52 ♂	62.7	1.65	73	231	63	3.67	5.31	−1.64
181	I – II	52 ♂	62.7	1.65	78	240	69	3.50	4.78	−1.28
172	II	56 ♂	74	1.91	72	239	52	4.59	3.83	0.76
172	II	56 ♂	74	1.91	80	282	54	5.22	4.46	6.76
146	II	49 ♂	77.9	1.90	74	303	27	11.22	9.85	1.37
184	II	44 ♀	95.6	2.07	94	358	40	8.95	9.39	−0.44
184	II	44 ♀	95.6	2.07	86	348	40	8.70	8.78	−0.08
188	II	47 ♀	93	2.0	58	195	51	3.82	5.28	−1.46
188	II	47 ♀	93	2.0	54	186	53	3.51	5.32	−1.81
196	II – III	35 ♀	68.2	1.70	90	258	48	5.37	4.21	1.16
166	II – III	69 ♀	69	1.73	32	184	58	3.2	3.68	−0.48
174	III	53 ♀	61.3	1.63	78	240	42	5.71	5.51	0.20
134	III	41 ♀	55.5	1.60	70	225	44	5.11	5.20	−0.09
139	IV	50 ♂	70	1.87	100	310	67	4.62	5.03	−0.41
157	IV	52 ♂	75	1.85	79	284	79	3.60	4.08	−0.48
157	IV	52 ♂	75	1.85	82	275	65	4.23	5.43	−1.20
183	IV	41 ♂	65.2	1.73	81	242	43	5.63	7.40	−1.77
186	IV	64 ♂	58	1.68	68	250	56	4.46	5.24	−0.78

Table 1.   *b. Normals.*

Case Nr.	Group	Age Sex	Weight Kg	Body Surf. Area m²	Pulse Rate	Oxygen Consumption ml./min.	A−V O₂ Diff. ml./l.	Cardiac Output l./min. Fick	Cardiac Output l./min. Hamilton	Difference l/min.
161	I	49 ♀	54.9	1.54	63	202	39	5.19	5.37	−0.18
190	I	30 ♂	61.5	1.79	58	213	46	4.63	5.64	−1.01
137	I	49 ♂	72	1.82	72	226	37	6.10	7.40	−1.30
176	I	26 ♀	48	1.50	81	208	29	7.17	6.96	0 21
177	I	17 ♀	68.4	1.74	88	192.5	23	8.36	7.29	1.07
165	I	19 ♀	50.6	1.51	96	177	27	6 55	7.72	−1.17

Table 1.   *c. Pulmonary Diseases.*

Case Nr.	Group	Age sex	Weight Kg	Body Surf. Area m²	Pulse Rate	Oxygen Consumption	A−V O₂	Cardiac Output Fick	Cardiac Output Hamilton	Difference
154	II	61 ♂	75.4	1.95	66	281	45	6.24	7.38	− 1.14
»	II	61 ♂	75 4	1.95	58	275	44	6.25	6.20	0.05
142	IV	58 ♂	53.4	1.67	98	316	40	7.90	7.00	0 90
»	IV	58 ♂	53 4	1.67	102	281	40	7 02	7 50	−0.48

Table I.   *d. Mitral valve lesions.*

Case Nr.	Group	Age Sex	Weight Kg	Body Surf. Area m²	Pulse Rate	Oxygen Consumption ml./min.	A—V O₂ Diff. ml./l.	Cardiac Output l./min. Fick	Cardiac Output l./min. Hamilton	Difference l./min.
155	I	41 ♀	56.3	1.62	79	293	39	7.51	7.60	— 0.09
160	I	25 ♀	56	1.65,	72	246	40	6.15	5.92	0.23
175	I	42 ♀	60.7	1.61	86	213	31	6.86	6.76	0.10
133	II	40 ♂	56	1.62	71	256	41	6.24	6.90	—0 66
162	II	54 ♀	66.7	1.77	76	235	39	6.03	6.40	—0 37
168	II	26 ♀	55.9	1.60	80	264	40	6.59	6 09	0.50
145	II	32 ♀	57.3	1.65	78	251	51	4.93	4.04	0.89
148	II – III	52 ♂	77.1	1.88	79	262	44	5.95	5.36	—0.59
191	II – III	41 ♀	66 ·	1.75	78	272	65	4.19	4.13	0.06
»	II – III	41 ♀	66	1.75	66	»	62	4.39	6.17	— 1.78
164	III	30 ♂	52.6	1.55	88	272	83	3.28	3.88	—0.60
»	III	30 ♂	52.6	1.55	82	289	86	3.36	3.89	—0.53
147	III – IV	50 ♀	48	1.46	61	245	63	3.89	5.12	— 1.23
116	III	36 ♀	74.9	1.82	101	240	38	6.31	6.65	— 0.34

Table I.   *e. Pregnancy.*

Case Nr.	Group	Age Sex	Weight Kg	Body Surf. Area m²	Pulse Rate	Oxygen Consumption ml./min.	A—V O₂ Diff. ml./l.	Cardiac Output l./min. Fick	Cardiac Output l./min. Hamilton	Difference l./min.
150		37 ♀	45.1	1.42	92	239	38	6.28	6.76	— 0.48
151		35 ♀	72.8	1.86	85	273	44	6.20	6.94	—0.74
153		30 ♀	62	1.68	68	230	33	6.96	5.63	0.33
»		30 ♀	62	1.68	—	240	30	8.00	9.36	— 1.36
159		30 ♀	61.7	1.68	64	268	41	6.53	5.70	0.83
»		30 ♀	61.7	1.68	69	283	38	7.45	6.00	1.45
158		20 ♀	63.2	1.68	86	270	23	10.4	8.50	1.90
150		37 ♀	45.1	1.42	84	195	36	5 42	5.86	—0.44

f. Miscellanous

Case Nr.	Group	Age Sex	Weight Kg	Body Surf. Area m²	Pulse Rate	Oxygen Consumption ml./min.	A—V O₂ Diff. ml./l.	Cardiac Output l./min. Fick	Cardiac Output l./min. Hamilton	Difference l./min.
156	I	24 ♀	56.2	1.58	108	253	27	9.35	9 52	— 0.17
182	II	24 ♀♂	43	1.45	140	228	21	10.86	7.74	3.12
181	I – II	52 ♂	62.7	1.65	73	231	63	3.67	5.31	— 1.64
»	»	52 ♂	62.7	1.65	78	240	69	3.50	4.78	— 1 28
126	II	25 ♀♂	60.1	1.77	68	234	37	6.32	5.41	0.91
169	II	44 ♂	77 8	1.94	118	311	57	5.45	6.22	—0.77
179	III	53 ♂	67.5	1.79	74	210	71	2.96	3.76	—0.50
179	III	53 ♂	67.5	1.79	78	210	62	3.39	4.26	—0.87
194	IV	53 ♂	101 8	2.22	54	310	58	5.34	5 40	— 0 06
194	IV	53 ♂	101.8	2 22	53	310	60	5 17	5.71	—0.54

Table 1.   *a. Hypertension.*

Case Nr.	Group	Age sex	Weight Kg	Body Surf. Area m²	Pulse Rate	Oxygen Consumption ml./min.	A—V O₂ diff. ml./l.	Cardiac Output l./min.		Difference l./min
								Fick	Hamilton	
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143	I	50 ♀	60	1.68	81	210	42	5.0	5.35	−0.35
152	I	45 ♂	57.5	1.74	64	237	42	5.65	6.16	−0.51
»	I	45 ♂	57.5	1.74	66	287	48	5.97	5.52	0.45
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149	I—II	48 ♂	70.1	1.84	69	227	44	5.16	6.68	−1.52
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172	II	56 ♂	74	1.91	80	282	54	5.22	4.46	0.76
146	II	49 ♂	77.9	1.90	74	303	27	11.22	9.85	1.37
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188	II	47 ♀	93	2.0	58	195	51	3.82	5.28	−1.46
188	II	47 ♀	93	2.0	54	186	53	3.51	5.32	−1.81
196	II—III	35 ♀	68.2	1.70	90	258	48	5.37	4.21	1.16
166	II—III	69 ♀	69	1.73	32	184	58	3.2	3.68	−0.48
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157	IV	52 ♂	75	1.85	79	284	79	3.60	4.08	−0 48
157	IV	52 ♂	75	1.85	82	275	65	4.23	5.43	−1.20
183	IV	41 ♂	65.2	1.73	81	242	43	5.63	7.40	−1.77
186	IV	64 ♂	58	1.68	68	250	56	4.46	5.24	−0.78

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Case Nr.	Group	Age Sex	Weight Kg	Body Surf. Area m²	Pulse Rate	Oxygen Consumption ml./min.	A—V O₂ Diff. ml./l.	Cardiac Output l./min.		Difference l/min.
								Fick	Hamilton	
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190	I	30 ♂	61.5	1 79	58	213	46	4.63	5.64	−1.01
137	I	49 ♂	72	1.82	72	226	37	6.10	7.40	−1.30
176	I	26 ♀	48	1.50	81	208	29	7.17	6.96	0 21
177	I	17 ♀	68.4	1.74	88	192.5	23	8.36	7.29	1.07
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»	II	61 ♂	75 4	1.95	58	275	44	6.25	6.20	0.05
142	IV	58 ♂	53 4	1 67	98	316	40	7.90	7.00	0 00
»	IV	58 ♂	53 4	1 67	102	281	40	7 02	7.50	−0 48

cardiac output in man. The dye injection method mainly covers the cardiac output during a short time, at most 10 seconds. It is thus possible that the value obtained is more influenced by the phases of respiration, while the Fick value necessarily represents the mean of the cardiac output during the respiratory cycles. The agreement between the two techniques was in our hand a little better than in the series of Hamilton et al. though the difference was not significant. This is of interest as our blood sampling method is considerable simpler, than the one described by Hamilton (1948), avoiding all mechanical devices and instead moving the receiving tubes by hand. In contrast to the determinations by Hamilton et al. our blood samples for both methods were taken within

2 minutes. The chances for a shift in basal state are thus less in our material than in theirs. Injection of the dye in the p.a. gives a dilution curve that almost approaches zero in most cases before recirculation appears, which makes the amount of extrapolation small and unimportant. If the dye is injected more peripherally in the venous system, it is diluted by more blood before sampling and a greater part of the curve must be extrapolated making the extrapolated part of the curve of more importance for the end result.

The sum of the standard deviations of the Fick and Hamilton method was 0.868 $+$ 0.074 l/min. i.e. 14.5 per cent. Calculations and estimates indicate that the errors of both methods are of the same order of magnitude, amounting to about 11 per cent.

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SEPARATE DETERMINATION OF THE BLOOD VOLUME OF THE RIGHT AND LEFT HEART AND THE LUNGS IN MAN WITH THE AID OF THE DYE INJECTION METHOD

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Hamilton, Moore, Kinsman and Spurling in 1931 developed a method of determining the cardiac output and intrathoracic blood volume according to principles given by Stewart in 1898. A dye was injected intravenously and its plasma concentration was determined in successive arterial blood samples taken with one or two seconds interval. The cardiac output may be calculated according to the formula

$$Co = \frac{60 \cdot D \cdot 100}{Cm \cdot T \, (100\text{-}H)}$$

where Co l/min. is the cardiac output, D ml the injected amount of dye, Cm ml/1000 the mean concentration of the dye during the first passage, T sec the duration of the first passage of dye and H the hematocrit. The volume passed by the dye is given by the formula

$$V = \frac{Co}{60} Tm$$

where V l. is the volume, Co l/min the cardiac output and Tm sec the mean circulation time.

$$Tm = \frac{C1 \cdot T1 + C2 \cdot T2 + Cn \cdot Tn}{Cm \cdot T}$$

where Cn ml/1000 is the dye concentration Tn sec after the injection.

After the dye has reached its maximal concentration the following concentration fall is logarithmic until the recirculation appears. If the concentration curve is plotted on semilogarithmic paper, the initial concentration fall follows a straight line. If this line is extrapolated downward to the indefinite one obtains the time concen-
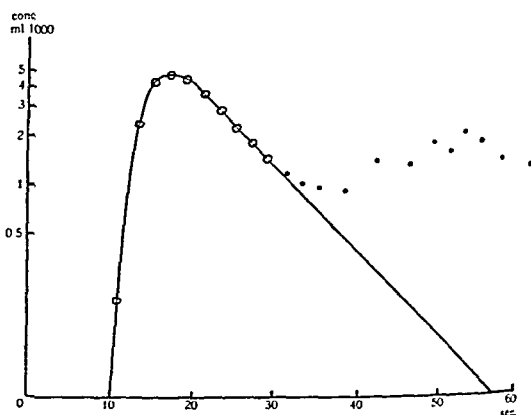
Fig. 1.

tration curve which should have appeared if no recirculation had occurred. This curve is used for the calculations. (Fig. 1.) The authors demonstrated the validity of the above formulas in circulation models and in animals.

The use of the injection method to calculate blood volumes in man has hitherto

been restricted to the determination of the intrathoracic blood volume with one exception. R. V. Ebert, C. Borden, H. S. Wells and R. H. Wilson in 1948 preliminary reported the determination of the blood volume between the pulmonary and femoral artery in normal cases and cases with heart disease. It is the purpose of this report to describe in detail the methods for determining the blood volumes in the right and left heart and in the lungs and report the values obtained in normals and in cases with different heart diseases. The principles and preliminary results have been reported earlier (Lagerlöf et al. 1948, 1949).

PROCEDURE

All patients reported in this paper have been studied in the postabsorptive state in the supine position. The pulmonary artery is catheterized through the left median vein and an indwelling arterial needle is placed in the right brachial artery. 30 minutes rest are allowed after this. Collection of expired air in a Tissot spirometer is then started. 4 or 5 ml of a 0.5 % solution of Evans blue dye is rapidly injected through the catheter in the pulmonary artery from an exactly calibrated syringe. As soon as the plunger reaches the bottom of the syringe it is withdrawn in order to empty the catheter from the dye left there. The catheter is then flushed with blood from the pulmonary artery. The dye and blood thus withdrawn are diluted with saline and the exact amount of dye determined. The injected dye is the amount in the syringe less the volume of the dye withdrawn. The dye is usually injected in 1 second. Half of this time is required to fill the catheter with dye. The calculations are corrected for these times.

Immediately before the dye injection the obturator is removed from the arterial needle and the blood is allowed to flow freely. Two stop-watches are started when the dye is injected and the seconds are counted out loud. The arterial blood is sampled in one or two seconds portions in previously heparinized ESR tubes according to Westergren. 20 tubes are placed close together in a wooden block that is moved by hand every or every other second. One of the watches are stopped when 20 tubes are filled in order to check that no delay of time has occurred. About ½ to 1 ml blood per second is usually obtained.

The whole procedure thus described takes less than 40 seconds. In cases in heart failure with slow circulation the blood sampling is prolonged to 90 seconds.

Immediately afterwards blood is sampled in heparinized syringes from the pulmonary artery and the brachial artery after which the collection of expired air is concluded. The blood samples are analyzed for oxygen in the Van Slyke apparatus and the air in the Haldane. The cardiac output is calculated according to Fick from these values.

Usually the vital capacity is registered immediately afterwards. The blood pressures in the pulmonary veins, pulmonary artery, right ventricle and auricle and in the brachial artery are recorded later with the Tybjærg-Hansen and Warburg electrical manometer, simultaneously with the ECG, PhCG and respiration. The details of this procedure are reported earlier.

Arterial blood samples are taken 5, 10, 20 and 30 minutes after the dye injection and analyzed for dye. The total plasma volume is calculated from this values.

All tubes are centrifuged and the plasma is removed with the aid of a micro-burett. The dye concentration is read in a ½ cm cuvett in a Coleman spectrophotometer with filter 620. If the amount of plasma is unsufficient it is diluted with uncoloured plasma.

The heart volume was determined teleroentgenologically according to Gidlund's modification of the Liljestrand, Lysholm, Nylin & Zachrisson (1939) formula with the patient sitting. The diameter of the aorta is determined according to Gidlund (1948).

PRINCIPLES AND CALCULATIONS

The blood volume of the heart.

Gidlund, Lindgren and Lagerlöf in 30 cases found a close correlation between the roentgenological heart volume during life and the heart weight at autopsy. The correlation coefficient was 0.93. 0.5 l corresponded to a weight of 0.290 kg, 1.91 to a weight of 0.710 kg. These figures can be used to estimate the total amount of blood in the heart. This will be $V \frac{kg}{1.05}$ where V is the volume in l, kg the heart weight in kg obtained from the regression line and 1.05 the specific weight of the heart muscle.

The magnitude of the errors inherent in this calculation is demonstrated by a case recently published by Nylin where the volume of the heart diminished extremely after administration of cedilanide. The roentgenological heart volume diminished from 1.92 to 1.12 l. The mean volume was 1.52 l. corresponding to a calculated heart weight of 0.575 kg and blood volume of 0.97 l. From our calculation the blood volume of the heart changed $\pm$ 0.29 l. against the recorded $\pm$ 0.40 l., i.e. the error in the calculation of the blood volume of the heart in this extreme volume change was about $\pm$ 0.11 l.

Nylin, Sällström & Ågren and Nylin have reported considerable decrease of the heart volume in the erect position as compared to in the supine. Larsson & Kjellberg 1948 were not able to verify this. Using an improved technique they found no or only slight change. The roentgenological heart volume in the erect position is therefore thought to be a good indication of the volume in the supine position.

The blood volume in the arterial tree.

The amount of blood in the arterial system that is included in the determination, when the blood is sampled from the brachial artery, must be substracted in order to get the blood volume of the heart and lungs. This would be simple if the arterial blood was collected in an unbranched aorta with constant diameter. The volume then would be the area of the aorta times the distance from the root of the aorta to the site of the arterial puncture.

The flow velocity and the dilution of the dye would be identical in all proximal parts of the arterial system if aorta is branching with unchanged total square area and if the peripheral resistance in all branches were proportional to their square ara. Furthermore they would equal the same values in an unbranched aorta with constant square area. The conditions are unchanged in the periphery as long as the linear flow velocity does not change due to friction or change of square when new branches are given off.

Changes in flow velocity due to friction can be disregarded as the mean pressure in the aorta and the larger branches is almost identical. Hamilton (1947) showed that aorta ascendens in dogs with high or normal blood pressure has a larger square area than the sum of all its branches. It is however also known that the sum of the square areas of the arteries increases peripherally. This increase in square area peripherally counteracts the decrease in square area in the proximal branches of aorta. The result is that the mean flow velocity from the root of the aorta to the site of puncture in the brachial artery approximately equals the velocity in the ascending aorta. The blood

volume in the proximal part of the arterial tree thus will be about the square area of the aorta times the distance from the aortic valves to the site of arterial puncture. The inherent error in this calculation is thought to be relatively constant and not invalidate a comparison between different individuals, as the relation between the different arterial branches and the peripheral resistance should not differ much in different individuals.

In order to estimate the magnitude of the errors in the above calculations, the blood volume in the left heart, and the proximal part of the arteries was determined in a case of auricular septal defect in a 48 year old woman. She did not show any symtom or signs of heart failure, the heart volume was within normal limits and there was a large shunt between the left and right auricle that could be demonstrated with the aid of blood gas analyses. The square area of the aorta was 3.70 cm² and the distance from the root of the aorta to the arterial puncture was 35 cm. The dye was injected through the catheter, the tip of which was placed in the left part of the left auricle. Fig. 2 shows the dilution curve obtained.

Fig. 2.

1.75 sec after the injection the dye appears in the arterial blood, holds a high concentration in 2 seconds and then rapidly descends logarithmically. 10 sec. later the concentration increases indicating the appearance of recirculation. The circulation time from the right auricle to the brachial artery in this case was 10 sec. Thus the curve was completed before any dye shunted to the right auricle could have appeared.

The cardiac output calculated from the curve was 3.64 l., which was 8.5 % higher than the value simultaneously obtained according to the Fick principle. A higher value should here been obtained, if dye had been shunted to the right auricle.

The volume passed by the dye was 235 ml. According to the calculations 130 ml was in the arterial tree and 100 ml in the left heart, i.e. twice the stroke volume. This result shows that blood volume in the proximal arteries is small and that our method for calculation gives reasonable values.

The determination of the square of the aorta is time consuming and includes unknown errors. For practical purposes we have therefore made the assumption that the blood volume in the arteries is proportional to the body surface area. An area of 1.60 m² corresponds to 130 ml. The values obtained in this way differ insignificantly from those directly calculated.

*

The blood volume in the lungs.

The volume of the blood in the pulmonary artery, the pulmonary capillaries and the pulmonary veins (= the pulmonary blood volume, PBV), the blood in the left heart and in the proximal part of the arterial tree is determined when the dye is injected in the
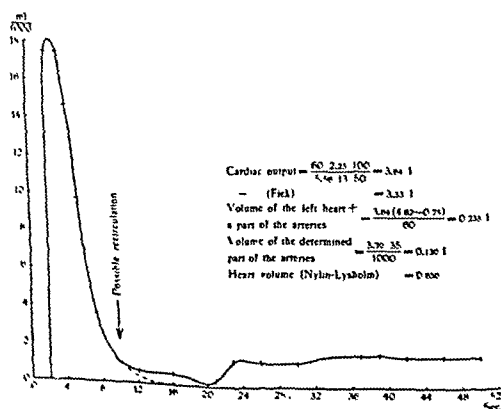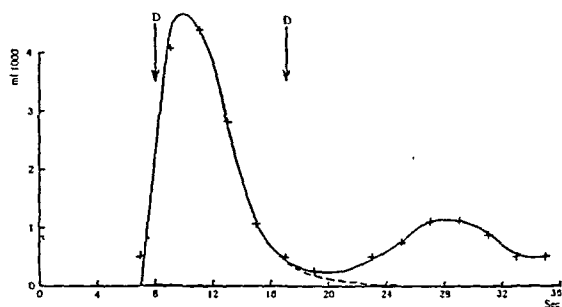
Fig. 3.   Arrows: begin and disappearence
of decholine taste.

pulmonary artery and the blood sampled
from the brachial artery. The amount of
blood in the proximal arteries can be calculated as shown above.

The blood volume of the left heart can not
be determined directly. It is about half of
the total blood volume of the heart or double
the stroke volume in persons without heart
enlargement. The different phases of the
cardiac cycle does not play any role as the
determined blood volume represents a mean
value. The volume changes of the heart
furthermore are small. During ventricular
systole the blood is flowing rapidly in the
auricles, in diastole this blood fills the
ventricles.

In cases with very large hearts the enlargement is due to a dilatation of both the right
and the left heart while in cases with slight
increase in size the enlargement is due to a
dilatation of only one side of the heart. The
assumption that half of the calculated blood
of the heart volume belongs to the left heart
thus does not give rise to large errors.

Fig. 3 shows the dilution curve from a
normal 41 year old woman pregnant in the
third month. 2.05 ml dye was injected in
the pulmonary artery. The dye appearance
time was 7 sec. This was 1 sec. shorter than

the circulation time determined with 5 ml 20
per cent decholin injected in the pulmonary
artery. It was 1 sec. longer than the circulation time from lung to ear obtained with
the oximeter according to Lindgren. The
curve is broader and the concentrations lower
than in Fig. 2, due to the larger amount of
blood that the dye is diluted in. After 17
sec. the recirculation begins to appear. The
disappearance of the decholin taste comes
much later than the decrease of dye concentration.

THE BLOOD VOLUME OF THE RIGHT HEART

The blood volume of the right heart can
be directly determined from double studies.
First the dye is injected in the pulmonary
artery and later, when the plasma dye concentration is constant, it is injected through
the catheter with the tip at the entrance of
the superior vena cava in the right auricle.

In Fig. 4 the dilution curves from a case
of isolated pulmonary stenosis in a 25 year
old woman are shown. The mean pressure
in the right auricle was increased, 10 mm Hg.
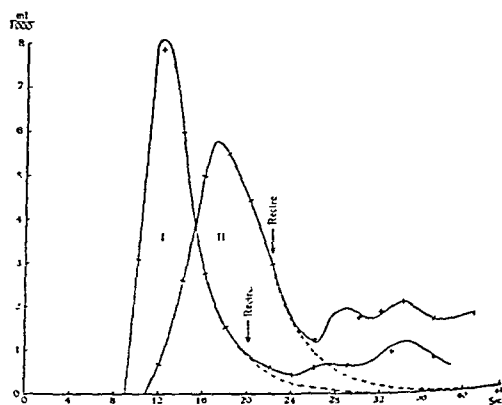
Fig. 4. Blood Vol. of the Right Heart = 1671 − 1371 =
300 ml. Total Heart Vol = 1000 ml (x-ray).

The systolic pressure in the right ventricle was 60 mm Hg, in the pulmonary artery 15. The arterial blood pressure was normal. The curve marked I was obtained when the dye was injected in the pulmonary artery, the curve marked II when the dye was injected in the superior vena cava. The appearance time in the latter was 2 sec. delayed. The summit of the curve was however 4,5 sec. delayed. The mean circulation time also was 4.5 sec. longer from the superior vena cava in comparison to the time from the pulmonary artery. When the dye was injected in the pulmonary artery only a small part of the curve must be extrapolated, when injected in superior vena cava considerably more.

RESULTS

The results of the determinations of the pulmonary blood volumes in cases with different cardiovascular conditions in different stages of decompensation is tabulated in Table I. The patients are grouped according to the classification of the New York Heart Association. The value for pulmonary blood volume varies from 511 to 1968 ml. There is no difference in the different stages of compensation.

Table II contains the average values. The PBV is lower in women than in men. This is the case in all groups. In pregnancy the PBV is still lower. In Table III the average value for PBV has been calculated related to cc predicted oxygen consumption in order to enable the comparison betwen all cases regardless of age, sex, weight and height. There is perhaps a little higher value in the decompensated cases (group III—IV), but the difference is too small to be statistically significant.

DISCUSSION

The method. That the described method gives a correct estimate for the circulating pulmonary blood volume is shown by the following reasoning.

Assume, that the lungs consists of an indefinite number of parallell systems, which arises from the main stem of the pulmonary artery and end in the left auricle and that the mixture of flows through the different systems here is immediate.

The volumes of the different systems $= V_1$, V_2, V_3, ... V_n l.
The flows through the different systems $= F_1$, F_2, F_3, ... F_n l/min.
The flows of dye through the different systems $= D_1$, D_2, D_3, ... D_n Gm.
The total flow or cardiac output is then ΣF_n l/min.

The total flow through the left auricle i.e. the cardiac output Co l/min. equals that through the aorta. It can be calculated from dye concentration time curves taken from the aorta, according to the formula $Co = \dfrac{\Sigma D_n \cdot 60}{Cm \cdot T}$, where $Cm =$ the mean concentration of dye during the time of the first passage of dye. This formula shows, that the obtained value for the cardiac output is independent of the volume the dye passes and of the mixing of dye from the different areas of the lungs. The only presumption for a correct figure of the cardiac output is that a correct dye concentration curve for the first circulation can be constructed.

The flow through the brachial artery is $C_{br} \cdot Co$. It is obtained from the dye concentration curve obtained from the brachial

Table 1. *Heart Volume, Pulmonary Blood Volume and Total Blood Volume in a'. Normals, b. Hypertensive Cardiovascular Disease, c. Mitral Valvular Lesions, d. Pulmonary Disease, e. Pregnancy and f. Congenital Heart Diseases and Cardioarteriosclerosis*

a) Normals.

Case Nr.	Group	Age	Sex	Weight kg	Body Surf. area m²	Determined blood vol., l.	Heart vol. cc.	Heart weight calc. kg	Calculated Blood vol. of the heart cc.	Estimated Blood vol. of proximal art cc.	Calculated pulmonary Blood vol. cc.	Total Blood vol. cc.	Puls Rate	P B V Per cent of total
137	I	49	♂	72	1.82	1.27	750	465	307	148	968	5000	72	19.2
161	I	49	♀	54.9	1.54	1.02	760	468	313	125	741	3810	63	19.4
170	I	23	♂	60	1.68	1.37	—	—	380	133	1014	6680	72	15.2
176	I	26	♀	48	1.50	1.650	670	440	251	142	1382	5470	78	25.2
177	I	17	♂	68.4	1.74	1.215	720	455	287	141	931	4740	88	19.7
190	I	30	♀	61.5	1.79	1.40	760	455	327	145	1091	5560	58	19.6
165	II	19	♀	50.6	1.51	1.01	—	—	273	123	751	4110	96	18.3

b) Hypertension.

Case Nr.	Group	Age	Sex	Weight kg	Body Surf. area m²	Determined blood vol., l.	Heart vol. cc.	Heart weight calc. kg	Calculated Blood vol. of the heart cc.	Estimated Blood vol. of proximal art cc.	Calculated pulmonary Blood vol. cc.	Total Blood vol. cc.	Puls Rate	P B V Per cent of total
140	I	47	♂	77.3	1.95	1.215	1000	535	491	159	810	6110	72	13.3
143	I	50	♀	60	1.68	1.38	930	515	439	137	805	5460	81	14.7
152	I	45	♂	57.5	1.74	1.79	835	490	369	141	1464	5170	64	28.4
163	I	26	♂	59	1.7	1.318	615	428	207	138	1076	7450	92	14.5
181	I–II	52	♂	62.7	1.65	1.06	740	460	302	134	785	5820	73	25.2
149	I–II	48	♂	70.1	1.84	1.613	1280	615	694	150	1464	6300	69	25.2
146	II	49	♂	77.9	1.9	2.21	1140	575	593	155	1590	5230	74	11.9
172	II	56	♀	74	1.91	1.00	950	525	450	155	620	5730	72	25.1
184	II	44	♀	95.6	2.07	1.780	840	490	373	168	1436	3870	94	11.3
188	II	47	♀	93	2.0	0.819	670	442	249	163	524	3840	58	25.1
166	II–III	69	♂	69	1.73	1.015	860	495	388	141	680	3500	32	17.6
196	II–III	35	♀	68.2	1.70	1.89	910	510	425	139	1.538	6660	90	40.1
174	III	53	♂	61.3	1.63	1.11	510	395	134	133	911	5060	78	26.1
157	IV	52	♂	75	1.85	1.628	1170	585	623	150	1166	4760	79	17.5
167	IV	34	♂	56.7	1.61	1.11	970	530	465	131	746	5930	122	14.8
183	IV	41	♀	65.2	1.73	1.350	940	520	445	141	986			20.7
186	IV	64	♂	58	1.68	1.72	930	515	439	137	1363		68	23.1
139	IV	50	♂	70	1.87	2.38[1]	1800	765	1072	152	1156	5960	100	17.7

c) Mitral Valve Lesions.

No.	Class	Sex	Age	C5	C6	C7	C8	C9	C10	C11	C12	C13	C14	C15
155	I	♀	41	56.3	1.62	1.51	930	515	440	132	1158	6070	79	19.1
160	I	♀	25	56	1.65	1.32	850	495	337	134	997	4950	72	20.2
175	I	♀	42	60.7	1.61	1.21	800	475	348	131	905	4700	86	19.3
145	II	♀	32	57.3	1.65	1.46	900	508	417	134	909	4530	78	20.1
162	II	♀	54	66.7	1.77	1.405	950	525	450	144	1036	4300	76	24.1
168	II	♀	26	55.9	1.6	1.22	770	470	322	130	921	4670	80	19.7
148	II–III	♀♂	52	77.1	1.88	1.375	1020	540	505	153	970	4870	79	19.9
191	II–III	♀♂	41	66	1.75	1.34	1300	622	707	142	844	5440	78	15.5
164	III	♀♂	30	52.6	1.55	1.44	1550	690	894	126	867	6140	88	14.1
147	III–IV	♀♂	50	48	1.46	1.36	830	485	368	119	1057	4020	61	26.3
187	IV		50	71.9	1.85	2.60	2080	845	1275	150	1812	6200		29.1

d) Pulmonary Diseases.

No.	Class	Sex	Age	C5	C6	C7	C8	C9	C10	C11	C12	C13	C14	C15
154	II	♀	61	75.4	1.95	1.64	830	485	368	158	1298	6920	66	18.8
171	II	♀	55	93.6	2.03	1.80	940	520	445	165	1190	7040	60	16.9
142	IV	♀	58	53.4	1.67	1.29	530	395	154	136	1078	6740	98	16.0
185	IV	♀	46	74.4	1.89	1.530	910	515	420	154	1166	6750	87	17.3

e) Pregnancy.

No.	Sex	Age	C5	C6	C7	C8	C9	C10	C11	C12	C13	C14	C15
138	♀	28	76.2	1.81	1.19	625	430	215	147	936	5190	109	18.1
141	♀	33	85.2	1.90	1.150	700	450	301	155	845	6120	95	13.8
150	♀	37	45.1	1.42	0.850	530	400	149	115	670	3650	84	18.4
151	♀	35	72.8	1.86	1.12	710	455	287	152	826	5890	85	14.0
153	♀	30	62	1.68	0.975	535	405	149	137	764	4530	68	16.9
158	♀	20	63.2	1.68	0.88	730	460	292	137	598	6200	86	9.7
159	♀	30	61.4	1.68	1.035	1240	605	664	137	567	4820	64	11.8
173	♀	37	62	1.63	0.906	495	390	124	133	511			

f) Miscellaneous.

No.	Class	Sex	Age	C5	C6	C7	C8	C9	C10	C11	C12	C13	C14	C15
156	I	♀	24	56.2	1.58	0.985	610	425	205	128	754	5770	108	13.1
169	II	♀	44	77.8	1.94	1.65	770	405	384	150	1308	11300	118	11.8
182	II	♀	24	43	1.45	0.950	485	390	104	118	780	3850	140	20.2
193	II	♀	49	65.3	1.60	1.88	630	430	231	130	1634	5200	132	31.5
179	III	♀	53	67.3	1.79	0.932	968	528	464	185	555		74	13.0
191	IV	♀	53	101.8	2.22	1.39	1020	540	505	180	957	7370	50 / 54	

T a b l e II. *Pulmonary Blood Volume in different stages of decompensation.*

	group	Men		Women	
		nr. of cases	mean ml	nr. of cases	mean ml
1. normals	I–II	3	1040	4	950
2. hypertension ..	I–II	6	1130	4	930
	III–IV	4	1150	4	968
3. mitral lesions ..	I–II			6	835
	III–IV	3	1195	2	950
1–3 total	I–II	9	1095	14	895
	III–IV	7	1170	6	964
	I–IV	16	1125	20	905
5. pregnancy	I–II			8	750

T a b l e III. *Pulmonary Blood Volume per ml pred. O_2 cons. in different stages of decompensation.*

	group	Men		Women	
		nr. of cases	mean ml	nr. of cases	mean ml
1. normals	I–II	3	4.53	4	4.90
2. hypertension ..	I–II	6	4.98	4	4.35
	III–IV	4	5.56	4	5.00
3. mitral lesions ..	I–II			6	5.11
	III–IV	3	5.53	2	5.30
1–3. total	I–II	9	4.83	14	4.83
	III–IV	7	5.58	6	5.10
	I–IV	16	5.15	20	4.91
5. pregnancy	I–II			8	3.85

artery according to the formula

$$C_{br} \cdot CO = \frac{C_{dbr} \cdot \Sigma D_n \cdot 60}{Cm_{br} \cdot T}$$

where C_{br} and C_{dbr} are constants and Cm_{br} the mean concentration of dye during the first passage. The necessary pre-sumption when this formula is used for calculation of the cardiac output is that $C_{br} = C_{dbr}$, i.e. that the amount of dye that flows through the brachial artery is proportional to the flow through the same artery. This is probably the case because the mixing of blood in the aorta should be complete.

The mean circulation time from pulmonary artery to left auricle for each pulmonary system is Tm_n sec. $= \dfrac{V_n \cdot 60}{F_n}$. The mean circulation time for both lungs, determined from the dye concentration curve from the left auricle will be

$$\frac{D_1 \, Tm_1 + D_2 \, Tm_2 + \ldots Dn \, Tm_n}{D_1 + D_2 + \ldots D_n}$$

The total lung blood volume Vc_1 l. calculated from the dye concentration curve in the left auricle is $\dfrac{Tmc_1 \cdot Co}{60}$ or

$$Vc_1 = \frac{\dfrac{D_1 \, V_1}{F_1} + \dfrac{D_2 \, V_2}{F_2} + \dfrac{D_n \, V_n}{F_n} \cdot \Sigma F_n}{\Sigma D_n}$$

If the dye is distributed to the different systems proportionally to the flow $D_n = C$. F_n, where C is a constant. Then $Vc_1 = \Sigma V_n$. This shows, that different ratios between vascular volume and flow in different parts of one lung does not invalidize the calculation of the pulmonary blood volume if the dye is evenly mixed with blood before the branching of the right or left pulmonary artery.

If the flow in the different systems is proportional to the volume i.e. $F_n = C \cdot V_n$, Vc_1 is again $= \Sigma V_n$. This shows, that when more of the dye is injected in the pulmonary artery of one lung the calculated volume of both lungs will be correct if the ratio be-

tween vascular volume and flow in the two lungs is the same.

The discussion above refers to the condition when the dye concentration time curve is taken from the left auricle. The mean circulation time to the brachial artery $Tm_{tot} = Tmc_l + Tmc_h$, where Tmc_h is the mean circulation time through the left heart and part of the arterial system.

The calculated total blood volume between pulmonary and brachial artery Vc_{tot}

$$= \frac{Tm_{tot} \cdot Co}{60} = Vc_l + \frac{(Tm_{tot} - Tm_l) Co}{60}$$

When Vc_l is correct Tm_l will also be correct and hence also Vc_{tot}.

It is evident that different distribution of dye to the right and left lung and different proportions between blood volume and flow through different parts of the lungs are not likely to give incorrect cardiac output figures or figures for the blood volume of the lungs, if the time-concentration curve for the first passage of dye can be correctly constructed. This implies that the concentration fall is semilogarithmic through its whole course. Model experiments by Hamilton et al (1931/32) have shown that this is the case.

A small part of the determined blood volume, in most cases below 10 per cent. extrapolated part of the dilution curve and may be erroneous due to the way the curve is extrapolated. This constitutes, however, only a small part of the calculated blood volume, in most cases below 10 per cent. The absence of a systematic difference between cardiac output values calculated from the dilution curve and these simultaneously obtained with the direct Fick method indirectly evidences that the extrapolation does not give rise to systematically errors (Hamil-

ton et al. 1948 ,Werkö et al. 1949). The relative importance of the extrapolated area in different conditions will be discussed in a separate paper.

The results. It is of interest that we have not been able to demonstrate any consistently increased PBV in the cases in decompensation as compared to normals. This is in contrast to the results of Ebert et al. They, however, included the blood volume of the heart in the PBV and consequently found an increased PBV in decompensated hypertensive cardio-vascular disease and in aortic insufficiency, conditions, that usually have a large left heart. The increase in PBV in their cases thus might be attributable to heart enlargement.

All our decompensated cases, however, were in a balanced state of decompensation. It is thus still possible that acute failure of the left ventricle may cause a temporary increase of the PBV, that is regulated when the state is in balance or regressing. This remains to be shown. Rapid digitalization in left ventricular failure has been accompanied by a decrease in the blood pressures in the pulmonary circulation and a marked increase in cardiac output without a significant change in the PBV in short time studies.

SUMMARY

1. The blood volume between the pulmonary artery and the brachial artery was determined in man from the dilution curve in the arterial blood of a known amount of Evans blue dye injected through a catheter in the pulmonary artery.

2. The total blood volume of the heart was calculated from a correlation between the roentgenological heart volume and the

heart weight. The amount of blood in the left heart was supposed to be the half of the total blood volume of the heart.

3. The amount of blood in the proximal part of the arterial tree was calculated by multiplying the square area of the aorta, determined roentgenologically, with the distance from the root of the aorta to the site of arterial puncture. The direct determination of the blood in the left heart and the proximal part of the arterial tree in a case of auricular septal defect showed that this way of calculation gives reasonable accurate values.

4. The amount of blood in the pulmonary artery, pulmonary capillaries and pulmonary veins (PBV) was calculated by subtracting the blood in the left heart and the blood in the proximal part of the arteries from the blood volume found.

5. The blood volume of the right heart was directly determined by injecting the dye first from the pulmonary artery and later from the superior vena cava and subtracting the blood volumes found.

6. The determined blood volume in the lungs varied between 511 and 1968 ml.

7. There was no consistent difference in PBV in different stages of decompensation or in mitral valvular disease as compared to hypertension.

ACKNOWLEDGEMENT

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EXAMINATION OF THE RENAL PLASMA FLOW BY MEANS OF PARA-AMINO-HIPPURIC ACID (PAH) USING ONE INTRAMUSCULAR INJECTION[1]

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The methods presently in use for examination of the kidney plasma and blood flow in man can scarcely by used routineously in clinical work. To some part this is due to the comparatively complicated technique which is necessary to keep the concentration of diodrast or PAH in plasma on a constant and suitable level. In 1947 Josephson published a method for the determination of diodrast clearance using one single intramuscular injection. This method represented a considerable advantage especially for the routineous use of diodrast in the determination of renal plasma flow. Other convenient methods for the injections of diodrast have also been published and are referred to in Josephson's paper.

However, the experience during the last year with PAH has shown that this substance is more suitable for the determination of renal plasma flow than the diodrast. It is more completely extracted during the passage through the kidneys (Warren et al. 1944), and the analysis is easier and more reliable.

By this reason series of experiments have been carried out trying to find a convenient way of administration of PAH facilitating its use in the determination of renal plasma flow. It soon was found, that an intramuscular injection of a 20 % solution could not be used. The injection was rather painful and the resorbtion was much too fast with the result that no constant plasma concentration was obtained.

Brun et al. (1947) published a technique consisting of one subcutaneous injection of a 20 % PAH-solution mixed with some NaCl. However, this method is not quite satisfactory. The subcutaneous injection is rather painful and the rate of resorbtion from the subcutaneous depôt is very varying, especially in cases with edema.

It was found that the pains following the injection of a concentrated PAH-solution could be avoided if the solution was mixed with a local anestetic. A mixture in suitable proportion with 2 % solution of dietylamino-2-6-xylididhydrochlorid (xylocain Astra) usually renders the injections completely painless. In a few cases a slightly disagreable feeling has been noticed.

It turned out, however, that after this way of injection the resorbtion of PAH took

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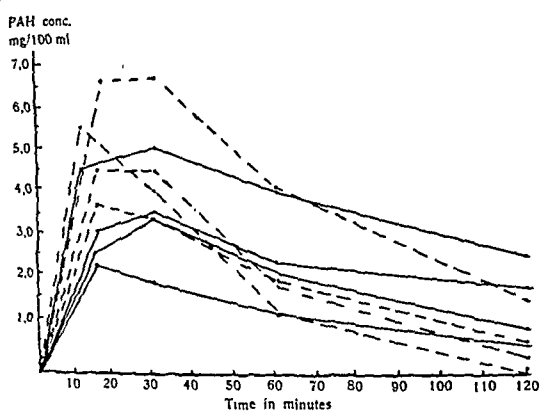
Fig. 1. Plasma PAH-concentration after i.m. injection without (— — — — —) and with (———————) adrenalin.

place too quickly with a too rapidly falling plasma concentration as the result. This difficulty was avoided if instead of the xylocain solution the PAH solution was mixed with a 2 % solution of xylocain-adrenalin. In the first experiments a mixture of 15 ml. 20 % PAH + 5 ml. 2 % xylocain-exadrin (Astra) was used. This small amount of adrenalin (0.06 mg) has no influence on blood-pressure or puls rate why it can be considered to be without any influence on filtration rate and blood flow.

Fig. 1 demonstrates the plasma concentration after one intramuscular injection in 4 human subjects, who got PAH mixed with xylocain-exadrin (drawn out curves) and 4 subjects who got PAH + xylocain without exadrin (dotted curves). In the latter cases quicker increase of the concentration and a higher maximum of PAH were obtained and the plasma concentration subsequently fell quicker so that it was not suitable for the determination of the plasma flow. With exadrin the concentration followed a more level curve and after two hours the concen-

tration was still suitable for a clearance determination. Later on it was found that a still better constancy was obtained by 0.3 ml. 20 % PAH + 0.05 ml. 2 % xylocain-exadrin (= 0.0006 mg adrenalin) per kg body weight. Only in one case the determination could not be fulfilled when this technique was used. In this case the plasma concentration was too low at the beginning and the concentration values became too low to allow reliable determination. Eventually the injection had happened to be partly intravenous.

The patients have been examined on an empty stomach or one hour or more after a very light breakfast. In the routine work the glomerular filtration has been determined with creatinine according to Rehberg 1926, as this method is more convenient than the inulin method and gives values only a little higher than the inulin clearance. The subjects got 3—6 g creatinine by mouth one hour before the examination was started. At the same time the subjects had to drink about 1 l. water. The intramuscular PAH injection was given half an hour before the examination was started. In the following this examination was carried out according to Smith et al. (1945) : 2—3 periods of 20—30 minutes were used according to the diureses. The blood samples were taken by a needle in one of the brachial arteries. By placing the needle in the artery before the starting of the experiment repeated puncturing was avoided.

Another advantage of letting the needle remain in the artery was that simultaneously with a clearance determination the cardiac output could be examined according to the modification of the method of Hamilton et al.

Table I. *Creatinine and PAH-clearance with the new injection technique.*

Case Nr.	Subject	Period	Diuresis ml./min.	Creatinine clearance ml./min.	Interpolated Plasma-PAH mg./100 ml.	Renal Plasma flow ml./min.	Creatinine clear. ×100 / PAH clear.	Diagnosis Remarks.
1.	♂ 30 Y. b. s. a. 1.88 m²	1. 2. 3.	1.6 7.7 14.4	207 207 184	2.25 1.60 0.80	975 924 954	21 22 19	Slight acute nephritis Normal blood pressure.
2.	♀ 51 Y. b. s. a. 1.79 m²	1. 2. 3.	0.5 2.5 6.3	108 126 131	0.70 1.00 1.15	567 485 480	20 26 27	Normal.
3.	♀ 34 Y. b. s. a. 1.62 m²	1. 2. 3.	1.1 1.0 1.3	114 73 116	1.65 1.35 1.10	532 452 600	21 16 19	Toxemia of pregnancy. Gravid mens VIII. Bladder probably not emptied at 1st period.
3.	Same case 14 d. post part. b. s. a. 1.55 m²	2. 3.	0.8 2.0	119 111	3.75 3 60	307 316	39 35	Delivered from a dead fetus Remaining Hypertension and proteinuria.
3.	Same case 1 mont post part. b. s. a. 1.55 m²	1. 2. 3.	6.8 9.3 5.8	111 141 105	5.65 4.45 3.55	340 364 273	33 39 38	Remaining Hypertension and proteinuria.
4.	♀ 38 Y. b. s. a 1.60 m²	2. 3.	5.9 6 6	104 127	3.70 3.00	322 410	32 31	Toxemia of pregnancy. Delivered from a dead fetus 1 month earlier. Remaining proteinuria
5.	♀ 35 Y. b. s. a. 1.75 m²	1. 2. 3.	3.6 3.8 5 1	152 150 151	3 95 3.20 2.40	500 645 605	30 30 25	Normal
6.	♀ 35 Y. b. s. a. 1.75 m²	1 2. 3	1.6 2.7 3.1	9 11 11	11.1 11.5 10 8	24 25 28	37 44 39	One week after toxemia of pregnancy with shock and oliguria. NPN 173 mg per cent.
6.	Same case 14 d. later	1 2 3	4 9 4.4 4.1	71 63 87	4.50 4.85 4.40	248 239 339	29 26 26	Remaining slight hypertension.
7.	♀ 28 Y. b. s. a. 1.83 m²	1. 2. 3	2.5 2.3 8.6	56 36 89	5 65 5.70 5.55	127 90 240	44 40 37	Chronic nephritis B. p. slightly elevated. NPN 43 mg 100 ml.
8.	♀ 27 Y. b. s. a. 2.03 m²	1. 2. 3.	4.6 4.8 5 6	245 249 108	6.1 5.4 4.8	836 1130 1090	29 27 18	Gravid mens IX Hypertension

Case Nr.	Subject	Period	Diuresis ml./min.	Creatinine clearance ml./min.	Interpolated Plasma-PAH mg./100 ml.	Renal Plasma flow ml./min.	$\dfrac{\text{Creatinine clear.}}{\text{PAH clear}} \times 100$	Diagnosis Remarks.
9.	♀ 17 Y. b. s. a. 1.58 m²	1. 2. 3.	7.0 4.3 16.0	90 66 110	4.45 4.25 3.65	288 214 462	36 31 24	Hypertension Peet's operation.
10.	♀ 29 Y. b. s. a. 1.74 m²	1. 2. 3.	1.3 5.5 9.4	153 167 140	1.80 1.20 1.10	783 804 672	20 21 21	Gravid mens III. Normal.
11.	♀ 32 Y. b. s. a. 1.62 m²	1. 2. 3.	1.4 1.1 0.8	185 200 171	2.75 .230 1.70	1031 990 990	18 20 17	Gravid mens X. Hypertension.

T a b l e II. *Clearance values when PAH was given with the new injection technique compared with the slow i.v. injection method.*

Case Nr.	Subject	Period	Diuresis ml./min.	Inulin clearance ml./min.	Interpolated Plasma-PAH mg./100 ml.	Renal Plama flow ml./min.	Filtration fraction	Diagnosis Remarks
12	♀ 29 Y. b. s. a. 1.48 m²	1 2 3	11.6 6.6 7.5	211 173 184	2.20 1.90 1.60	740 755 876	29 23 21	Normal. PAH given with the i. m. technique.
12	1. week later Same case	1 2	6.1 8.5	113 104	2.60 2.35	758 860	15 12	PAH given with the slow i. v. technique.

(1931—32), published by Lagerlöf et al. (1949). The determination of the cardiac output simultaneously with the examination of the renal plasma flow is of great importance. It is more correct to correlate the the renal plasma flow to the cardiac output than to the body surface area. This correlation will be further emphasised in a following paper.

If inulin is used instead of creatinine the clearance determination can easily be fol-lowed by an examination of the tubular excretory capacity using one large intra-venous injection as shown by Josephson (1947) with diodrast.

Table I shows the results of 14 examin-ations of creatinine and PAH clearance on 11 human subjects. 3 were normal cases. The others were cases of nephritis, arterial hypertension, remaining kidney troubles after toxemia of pregnancy. 5 of the patients were pregnant which caused some difficulties in

emptying the urine bladder. The creatinine clearance in proportion to the PAH clearance has been recorded in order to indicate if the emptying of the urine bladder has been complete or not. In the case 6 which suffered from a severe kidney damage the "depression limit" for the PAH concentration was probably decreased and consequently it is probable that the concentration values obtained were above this limit. Thus it is probable too that selfdepression of the PAH clearance has occurred and the figure i the plasma flow become too low.

In Table II a case is demonstrated which the PAH clearance was determin at two instances: in one the new techniqi was used, in the other the continuous i travenous technique. The results are i comparatively good conformity to each othe In this case the glomerular filtration w; determined with inulin.

SUMMARY

A new technique is described for the examination of the renal plasma flow with para-amino-hippuric acid (PAH) instead of the slow intravenous injection previously used. The PAH is given in one intra-muscular injection together with a loc anestetic and adrenalin. The method i simple and easy to carry out and seems t give satisfactory results.

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THE DIAGNOSTIC AND PROGNOSTIC SIGNIFICANCE OF PLASMA PROTHROMBIN AND FACTOR V LEVELS IN PARENCHYMATOUS HEPATITIS AND OBSTRUCTIVE JAUNDICE[1]

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INTRODUCTION

Since Warner Brinkhous and Smith (1937) first suggested that estimation of the plasma prothrombin level could be used in the assessment of liver function, much work on this problem has been published. The investigations have been based on the estimation of the plasma prothrombin level in liver disease and obstructive jaundice and on the effect of vitamin K administration in such circumstances.

The following facts seem well established. Prothrombin is formed in the liver and damage to the liver parenchyma is accompanied by a lowering of the plasma prothrombin level. In such cases the response to vitamin K therapy is usually poor. In obstructive jaundice the prothrombin deficiency which occurs is due to faulty absorption of vitamin K from the intestine. In these cases the prothrombin level is restored to normal by adequate vitamin K therapy.

Earlier investigations have depended essentially on the estimation of the plasma prothrombin by Quick's method or some modification of this. Following the discovery of the fifth coagulation factor (factor V) and the part which it plays in the clotting-process, it became obvious that these methods did not allow of the exact quantitative estimation of the plasma prothrombin (Owren 1944). The so-called "prothrombin-time" estimated by Quick's method depends as much on the concentration of factor V as the plasma prothrombin level.

The two-stage method of Warner, Brinkhous and Smith is no more reliable for the results are too low if the factor V level is reduced. (Owren 1947 p. 307). A more precise method of estimating the prothrombin level is therefore required.

Hitherto there have been no studies of the factor V level of the blood in disease of the liver and bile-passages. Earlier experimental and clinical attempts to clarify the position have given inconclusive results through deficiencies of method.

In the work which follows quantitative investigations of the prothrombin and factor V levels in hepatitis and obstructive jaundice are recorded. The estimations were carried out by methods previously described, which

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allow accurate determination of both these coagulation factors. (Owren 1947, 1949.)

In the method for estimation of factor V, a plasma from a case of parahemophilia, lacking this factor, has been applied instead of the prothrombin and fibrinogen reagents described. After the discovery of an other new clotting factor (Owren & Bjerkelund 1949) it has now been shown that this procedure at present gives the only reliable method for the assay of factor V. In parahemophilia the concentration of the new factor is normal.

The investigation confirms, in the main, earlier suppositions about the behaviour of the prothrombin level. It shows further that an accurate estimation of factor V and prothrombin is a valuable help both in diagnosis and prognosis in these conditions.

In the following account the entire material is not published, for the variations of the prothrombin and factor V levels follow a characteristic course for each particular group of diseases. These courses are illustrated by accounts of typical cases of each disease.

RESULTS

1. Acute hepatitis, mild or moderate degree

The typical findings are as follows: *Factor V* is either normal or moderately increased up to 120—130 %. The concentration then falls slowly to the normal level coincident with recovery.

The prothrombin level begins to fall a little before or at the same time as jaundice appears. It falls as a rule to values between 40 % and 80% of normal and increases thereafter slowly to the normal level at the same time as the jaundice clears.

Synthetic vitamin K (2-methyl, 1—4 naphthoquinone) intravenously produces a rise in the prothrombin level of 10—20 %. This rise is transient.

Case 1: A 45 year old woman had become increasingly jaundiced in the 5 days preceeding admission. Icteric index 48. Liver slightly enlarged, edge palpable 1½ fingers below costal margin, slightly tender. Urin: bilirubin ++, urobilinogen increased. (Schlesinger+ 1/50.) Takata-Ara reaction+. Thymol turbidity test 0.28 units (normal < 0.10). Serum alkaline phosphatase normal (6.8 units). Serum iron 197 γ %. Serum albumin 2.6 g %. Serum globulin 3.2 g %. Uncomplicated course. The variations of the prothrombin and factor V levels and the effect of vitamin K administration are shown in Fig. 1.

2. Acute hepatitis, severe degree

In the more serious cases of acute hepatitis, besides the fall in the prothrombin level to 50 % (as a rule) or lower, the level of factor V also falls. The reduction is proportional to the degree of damage of the liver parenchyma and can fall to as low as 60 % of the normal value. Such patients may, however, make a complete recovery. Improvement and recovery are accompanied by a slow increase both of factor V and prothrombin.

If, on the contrary, the concentration of factor V falls below 50 %, a malignant hepatitis develops as a rule, which takes either a rapidly fatal course or passes on to a state of chronic hepatitis. Vitamin K administration has no effect on the levels of prothrombin and factor V, when these are low.
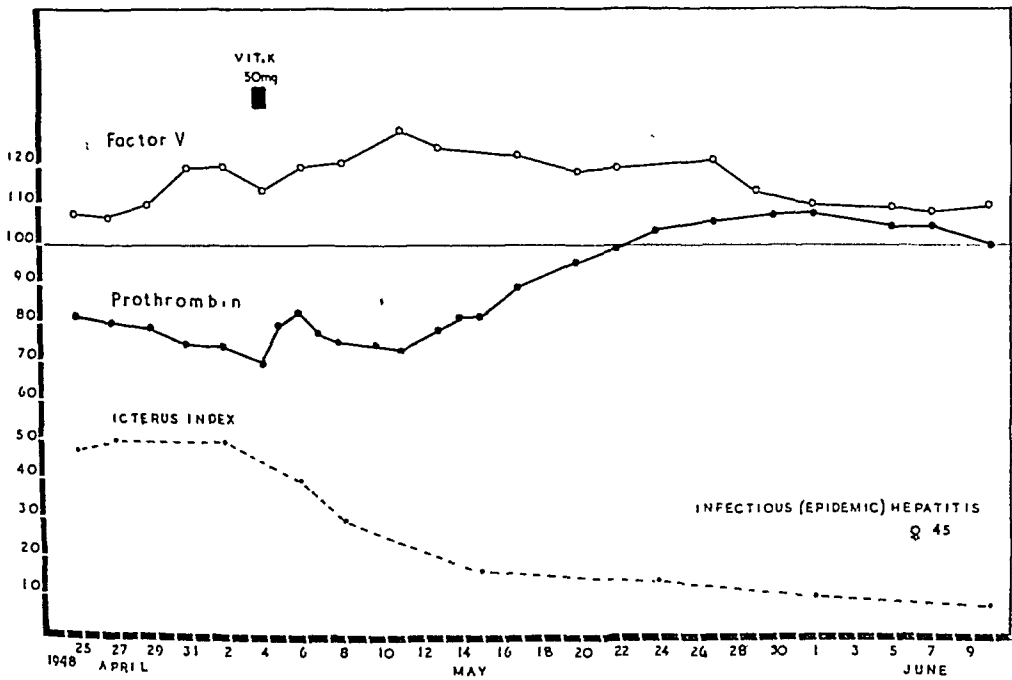
Fig. 1.
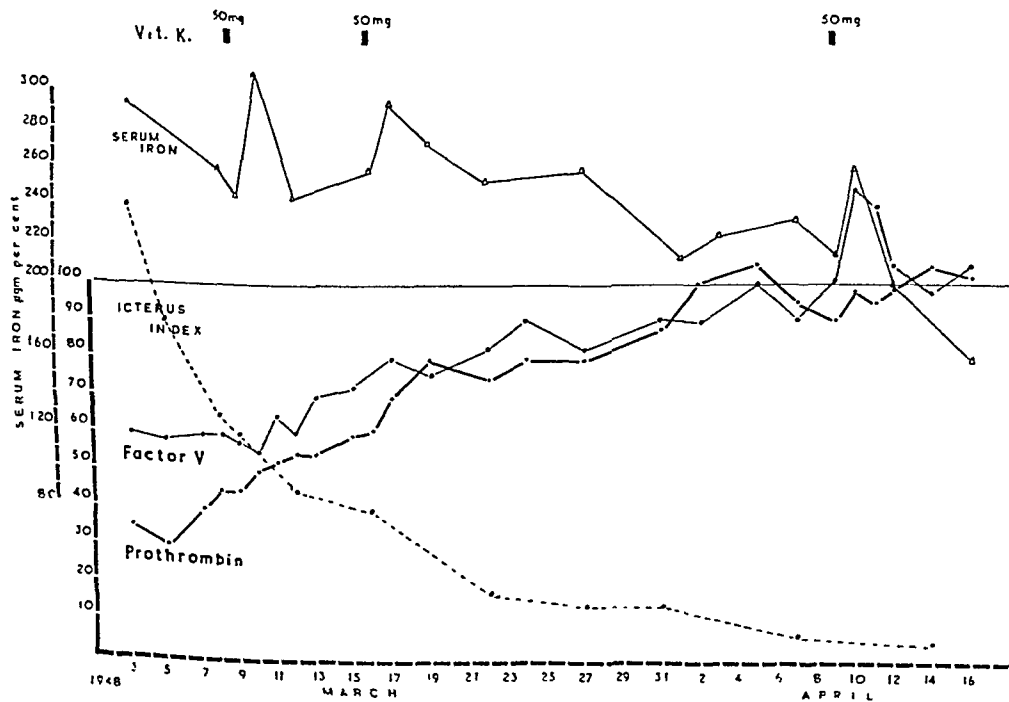
Fig. 2.
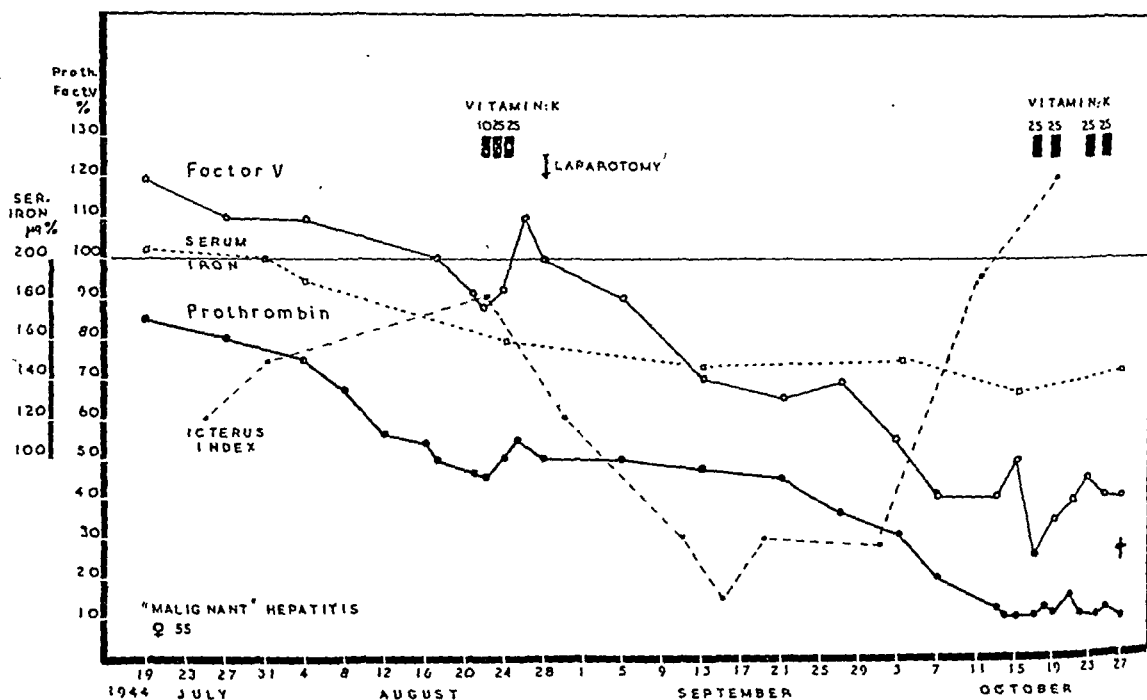
Fig. 3.

Case 2. A 24 year old man with jaundice of 14 days' duration before admission to hospital. On admission, the icteric index was 120. Liver edge palpable 1 finger-breadth below the costal margin, slightly tender. Urin: bilirubin + +, urobilinogen increased (Schlesinger + 1/50). Thymol turbidity test 0.30. Takata-Ara reaction +. Serum iron 295 γ %. Serum albumin 4.0 g%. Serum globulin 2.7 g %. Uncomplicated course. The data of the prothrombin and factor V levels are shown in Fig. 2

3. *Malignant hepatitis*

If the hepatitis takes a malignant course, a steadily progressive reduction of prothrombin and factor V occurs. The level of prothrombin falls earlier and remains always lower than that of factor V. In individual cases, there are shorter or longer periods where the levels of factor V and prothrombin are relatively constant. Occasionally a moderate transient increase in the levels occurs, which is then followed by a renewed fall. Early in the disease vitamin K administration exerts a transitory effect on the prothrombin concentration as in acute hepatitis. Later in the course of the disease when the levels of prothrombin and factor V are lower, vitamin K administration is without effect. (The malignant form of hepatitis was comparatively common in Scandinavia during and after the recent war, especially in women over 50.)

Case 3. A 67 year old woman. (Her sister, aged 48 died of malignant hepatitis in the
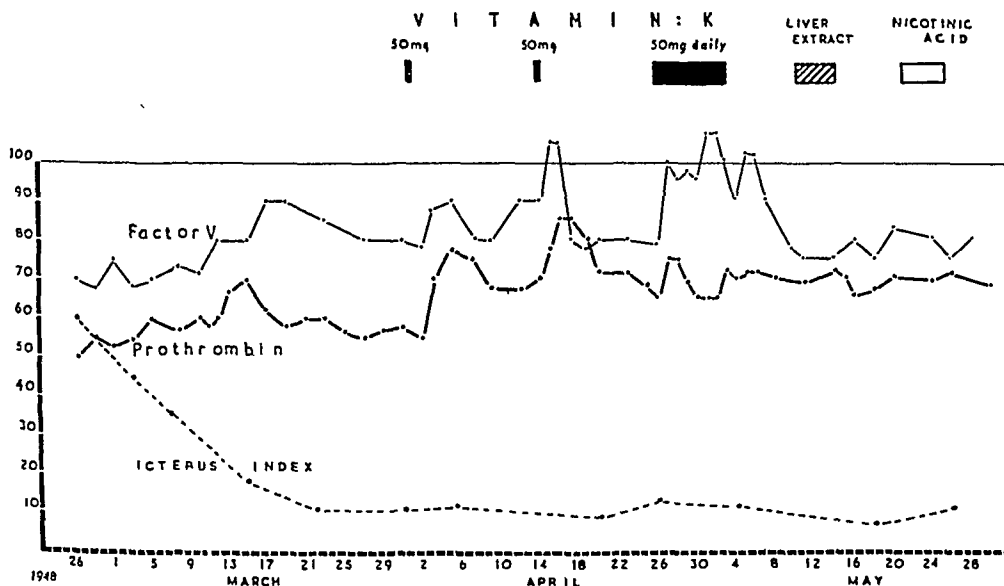
Fig. 4.

ward 3 months before the pasient was admitted.) This pasient fell ill in May 1944 with the usual symptoms and signs of acute hepatitis. After she had been confined to bed for 5 weeks, her condition improved and the jaundice decreased, but after an interval of 14 days she relapsed, her condition worsened and she was admitted to hospital.

On admission the liver was enlarged to 3 finger-breadths below the costal margin. The icteric index and serum iron are shown in Fig. 3. The urine contained bilirubin and the urobilinogen content was increased (Schlesinger + 1/30). Thymol turbidity test +. Takata-Ara reaction +.

In the course of 2 months the patient's condition grew steadily worse with signs of increasing liver damage, falling serum albumin, positiv Takata-Ara reaction and steadily falling levels of factor V and prothrombin (see Fig. 3).

Cholangiography done in the course of an exploratory laparotomy showed that there was no obstruction of the bile passages. After the operation there was a rapid clearing of the jaundice, but the rest of the symptoms continued as before. A haemorrhagic tendency appeared through lack of prothrombin and factor V, increasing symptoms of liver failure developed and the patient died in cholaemia.

4. Chronic hepatitis

In the development of a chronic hepatitis the plasma concentrations of prothrombin and factor V are characteristically reduced and remain continuously at subnormal levels.

Case 4. A woman aged 51, who had a third attack of jaundice within 4 months. The liver was enlarged to 2 fingerbreadths below the costal margin. Thymol turbidity test 1.08. Takata-Ara reaction +.
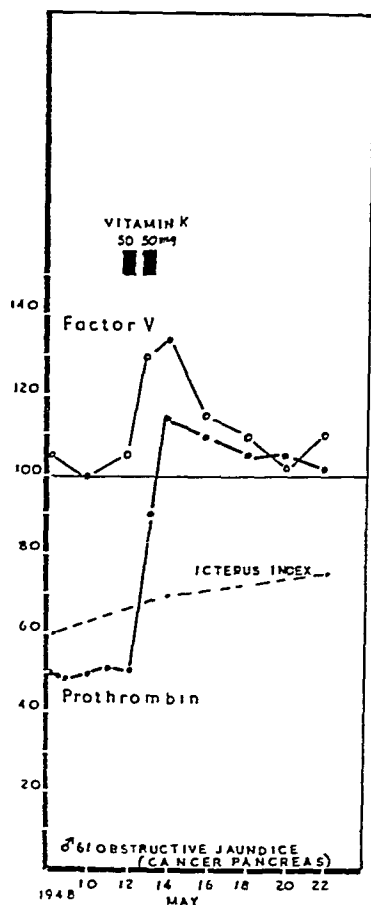
Fig. 5 a.
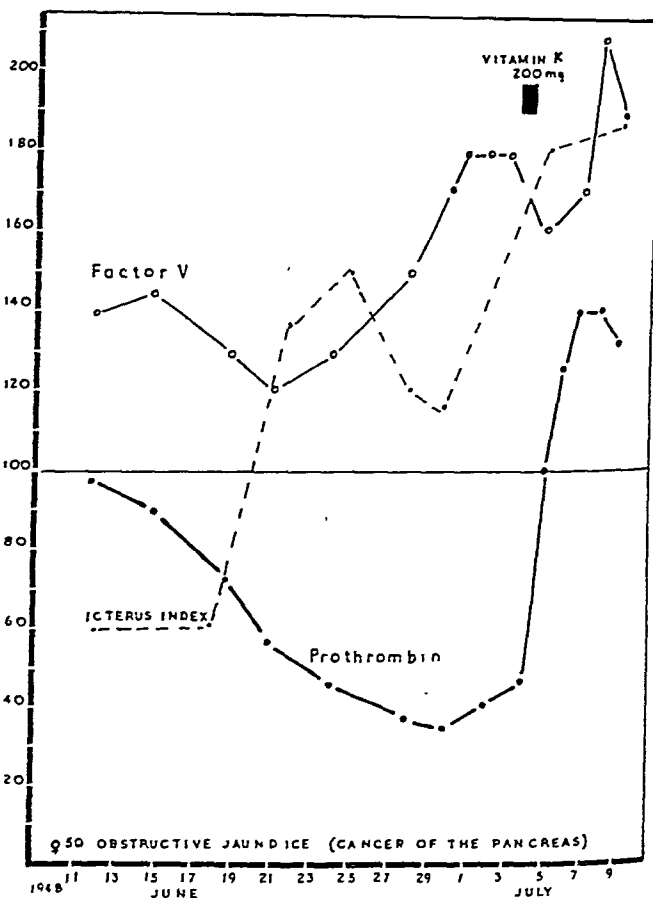
Fig. 5 b.

Serum alkaline phosphatase normal (4.7 Bodansky units). Serum albumin 3.4 g %. Serum globulin 5.9 g%. Serum iron 230 γ%. The icteric index fell to normal but the concentrations of prothrombin and factor V continued unaltered at their reduced levels. (See Fig. 4.) Vitamin K administration had only a small and transient effect. The serum albumin remained low at about 3 g % in the further course of the disease while the serum globulin continued at a raised level of about 6 g %. The Takata-Ara reaction remained constantly positive. Further observation showed the development of a chronic hepatitis with progressive fibrosis.

5. Obstructive jaundice

In obstructive jaundice the concentration of factor V is normal or slightly increased, but never reduced. In isolated cases the increase is considerable, (see Fig. 5 b). If the obstruction is relatively complete, a progressive reduction of prothrombin occurs. The parenteral administration of vitamin K (50
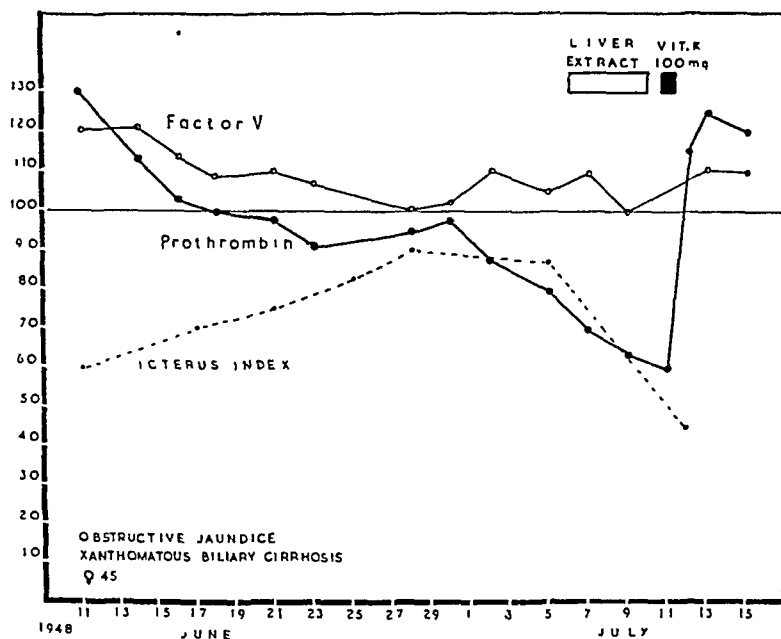
Fig. 6.

—100 mg) produces a rapid response; the prothrombin level is raised to normal levels or higher in the course of 24—48 hours.

Case 5. (Fig. 5 a.)   A man of 61 who had a steadily deepening jaundice in the 4 weeks prior to admission. The prothrombin level was lowered; the factor V level slightly raised.   After 50 mg vitamin K intravenously, the prothrombin concentration was increased from 50 % to 90 % in 24 hours and was 110 % after 48 hours. At operation, a carcinoma of the pancreas was found.

Case 6. (Fig. 5 b.)   A woman of 50 who had had a moderate jaundice for 14 days before admission. Her prothrombin concentration when admitted was 100 % and her factor V concentration 140 %. The icteric index was 60. The jaundice rapidly deepened. The stools were pale. There was no urobilinogen in the urine or faeces, a sign of complete obstruction.   The prothrombin

concentration fell in the course of 6 weeks to about 30 %.   The factor V level was increasing. Vitamin K administration gave a quick response and the prothrombin concentration increased to 100 % in the first 24 hours, rising to 140 % in the succeeding 24 hours.   A carcinoma of the pancreas was found at operation.

In both cases, the Thymol turbidity test and the Takata-Ara reaction were negative; the serum iron and serum proteins were within normal limits while the serum alkaline phosphatase levels were 19 and 7 (Bodansky units) respectively.

Case 7. (Fig. 6.)   A woman of 45 who showed a clinical syndrome of xanthomatous biliary cirrhosis.   She had had a jaundice fluctuating in intensity for the past 2 years prior to admission and had shown an increased bleeding tendency, especially ecchymoses and petechiae on repeated occasions

during this time. The bleeding tendency disappeared rapidly in response to vitamin K therapy. Shortly before admission she had had 10 mg vitamin K parenterally, and when admitted her prothrombin level was 130 % and her factor V level 120 %. There were extensive xanthomatous deposits in the skin. The liver and spleen were enlarged. The total serum cholesterol was enormously increased (1000—1200 mg%). The neutral fat in the serum was low and the serum was transparent and not creamy despite the great increase of cholesterol. A gradual reduction of prothrombin to 60 % took place in the course of 2—3 weeks. The factor V concentration remained unaltered. Vitamin K administration produced a rapid rise of prothrombin to values well above normal in 24 hours. (See Fig. 6.) The Takata-Ara reaction, Thymol turbidity test, serum protein concentrations and the galactose tolerance test all gave normal results. The jaundice in this disease is obstructive in type. Many years are required for the development of hepatic fibrosis and parenchymatous failure.

6. *Vitamin K deficiency in intestinal disease*

In vitamin K deficiency due either to diminished synthesis in the gut or diminished absorption, a reduction of the prothrombin level occurs while factor V always remains unaltered. Administration of vitamin K produces an increase of prothrombin to normal levels. In vitamin K deficiency due to intestinal disease the response to vitamin K therapy is slower than in obstructive jaundice. 4—5 days are often required before the prothrombin reaches normal levels despite the administration of large doses of vitamin K parenterally.

Case 8. A woman of 42 who had had clinical symptoms of idiopathic steatorrhœa with fatty stools, iron-deficiency anaemia and hypocalcaemia with frequent attacks o tetany for 15 years. On a diet containing 100 g fat, the daily output in the faeces was 35 g. The nitrogen excretion was normal Serum calcium 5—6 mg%. Serum phosphorus 3.7 mg%. Prothrombin level reduced Factor V level normal. Vitamin K therapy produced a slow rise of the prothrombin level to normal. (See Fig. 7.)

DISCUSSION AND CONCLUSIONS

The investigations recorded above show that the concentrations of prothrombin and factor V in the blood are reduced in diseases of the liver parenchyma which affect its function. The degree of reduction seems to follow fairly closely the degree of liver damage.

In the course of development of a liver disease, the plasma prothrombin level falls sooner and more severely than the factor V level.

In obstructive jaundice, the prothrombin is reduced, while the concentration of factor V in the plasma is normal or moderately raised. In vitamin K deficiency from intestinal disease the prothrombin is reduced and the factor V level normal.

These findings suggest that factor V, like prothrombin, is formed in the liver or that its formation is dependent on normal liver function. Factor V is, however, independent of vitamin K.

In obstructive jaundice the parenteral administration of synthetic vitamin K (2-methyl, 1.4-naphthoquinone) in large doses (50—100 mg) produces a rapid increase of

the prothrombin concentration to normal or super-normal levels.

In vitamin K deficiency due to intestinal disease, the increase of prothrombin following vitamin K therapy, takes place more slowly.

In moderate damage to the liver parenchyma (acute and chronic hepatitis), vitamin K produces a moderate and transient rise in the plasma prothrombin concentration. In severe damage to the liver parenchyma, the administration of vitamin K has no effect on the prothrombin concentration.

In cases where vitamin K therapy produces an increase in the prothrombin level, the level of factor V is often transiently raised at the same time.

The investigations seem further to show that a reduction of factor V results from relatively serious damage to the liver parenchyma. If the factor V level falls to below 50 % of normal, the prospects of recovery are small. If the factor V concentration in parenchymatous hepatitis remains for long periods at sub-normal levels without signs of increasing, this points to the development of chronic hepatitis. Along with this, there is then found a moderate hypo-prothrombinaemia which is refractory to vitamin K therapy.

A steadily progressive reduction of factor V over fairly long period is a serious prognostic sign, which indicates the development of a malignant hepatitis with fatal termination. Concurrent with this there are a considerable reduction of the prothrombin level and no response to vitamin K therapy.

The appearance of a bleeding tendency in obstructive jaundice and vitamin K deficiency is due to a simple lack of prothrombin.
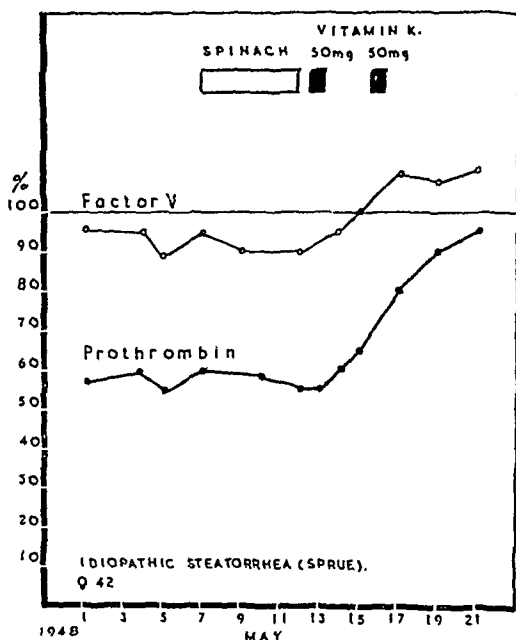
Fig. 7.

The bleeding tendency in diseases of the liver parenchyma is due to the combined effects of prothrombin and factor V reduction.

The investigations show that the exact quantitative determination of prothrombin and factor V and the effect on the prothrombin level of vitamin K administration can be of great value both in the diagnosis and prognosis of diseases of the liver and bile-passages.

SUMMARY

Investigations of the blood-concentrations of prothrombin and factor V in various liver diseases, in obstructive jaundice and vitamin K deficiency from other causes are recorded.

From the investigations, the following conclusions can be drawn:

1. The formation of factor V is dependent on normal liver function. The concen-

tration of factor V decreases with increasing damage to the liver parenchyma.

2. The formation of factor V is independent of the supply of vitamin K.

3. In parenchymatous hepatitis, a concentration of factor V under 50 % of the normal value is a bad prognostic sign. A steadily falling factor V concentration indicates the development of a malignant hepatitis with fatal ending. Persistently sub-normal values point to the development of chronic hepatitis.

4. In obstructive jaundice factor V is normal or increased. In vitamin K deficiency from intestinal disease factor V is normal.

5. The blood prothrombin concentration is reduced both in affections of the liver parenchyma and vitamin K deficiency (Obstructive jaundice and intestinal disease.)

6. In obstructive jaundice without damage to the liver parenchyma, the parenteral administration of vitamin K produces an increase of the prothrombin level to normal or super-normal values in 24—48 hours. Where the hypoprothrombinaemia is due to intestinal disease the increase of the prothrombin level in response to vitamin K therapy takes place more slowly as a rule.

7. In milder degree of damage to the liver parenchyma, vitamin K therapy produces a moderate and transient rise in the prothrombin level. In severe damage to the parenchyma, the administration of vitamin K is without effect.

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THE SERUM PROTEIN IN PATIENTS WITH HIGH GASTRIC CARCINOMA BEFORE AND AFTER TOTAL GASTRECTOMY

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The protein nutrition in patients suffering from gastric carcinoma still presents many clinical and pathophysiological problems. It has been demonstrated by a series of investigations that many of these patients have hypoproteinemia in various degrees which stands in no definite relation to their clinical status, though it is most marked in emaciated and cachectic patients. The causal relations have not by far been elucidated even if some general pathogenetical factors have been established, such as deficient diet, hemorrhage, increased protein metabolism, and further metabolic abnormalities which prevent the synthesis of serum protein.

The therapeutical problems which arise in the preoperative as well as the postoperative period partly bear the stamp of this uncertainty as far as the pathogenetical relations are concerned. Unsurmountable difficulties are often met with, therefore, in the treatment of this hematological symptom of abnormal protein balance. When such patients also have been subjected to total gastrectomy, who in our experience frequently present symptoms of considerable nutritional deficiency, an additional complicating factor is the part taken by the stomach in the protein digestion and its effect on the production of hemoglobin.

THE MATERIAL

50 patients with high gastric carcinoma have been examined with a view to the state of serum proteins in the preoperative and postoperative period after total gastrectomy. For the sake of keeping control of their nitrogen balance the nitrogen excretion in urine and faeces has been examined and simultaneously the peroral and parenteral protein intake has been controlled. Further systematic examination has been carried out of hemoglobin, erythrocyte and hematocrit values, together with their fluid balance.

The value of total proteins in serum in these patients has varied between 4.48 and 8 per cent on admission (Fig. 1). In the great majority of those patients who have demonstrated normal or almost normal values, it is found that when they have reached fluid balance the concentration falls, and as simultaneous anamnestic information is present of swallowing difficulties and considerable loss of weight, it is reasonable to connect this with the state of dehydration demonstrated. In spite of the low protein values edema have not been observed in any of these patients on admission, a circumstance which must also be ascribed to the relatively low voluntary fluid intake. It has been noticed, namely, that when these
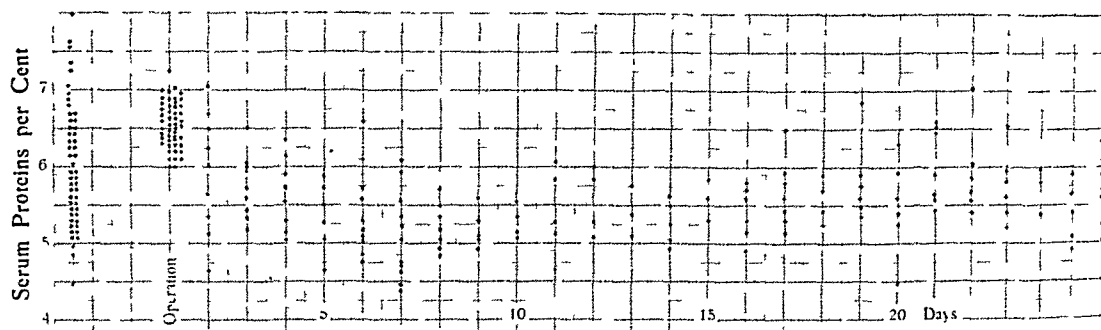
Fig. 1.
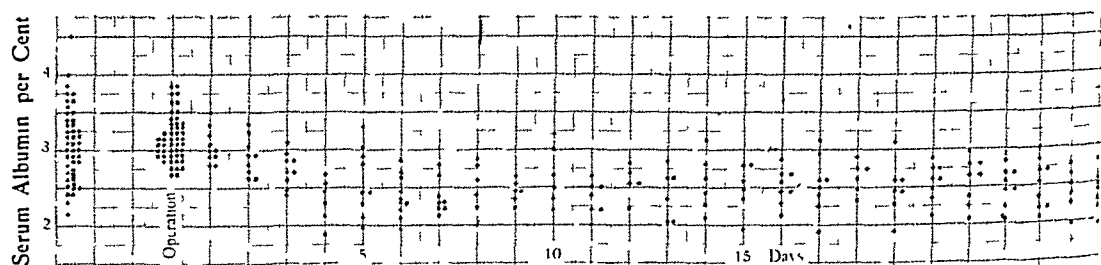
Fig. 2.
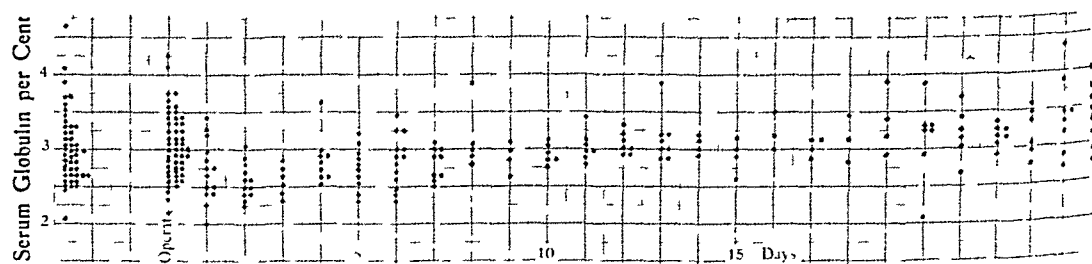
Fig. 3

patients receive an adequate supply of fluid, they easily develop edema if the hypo-proteinemia present is not treated at the same time

The albumin fraction in serum in these patients has been remarkably low, varying between 1.84 and 3 96, an average value of 2 98 (Fig. 2). In a number of these it has been lying below the edema limit, though edema have not been demonstrable. During the preoperative treatment with addition of blood and plasma, and also concentrated nutritional preparations perorally, the values have risen somewhat, though not in relative proportion to the total protein values Relatively few patients, therefore, have come for operation with normal albumin values in spite of the fact that the great majority of
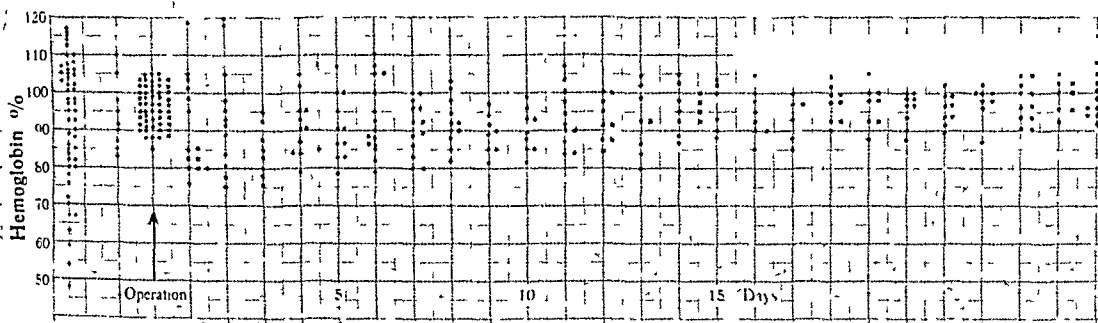
Fig. 4.
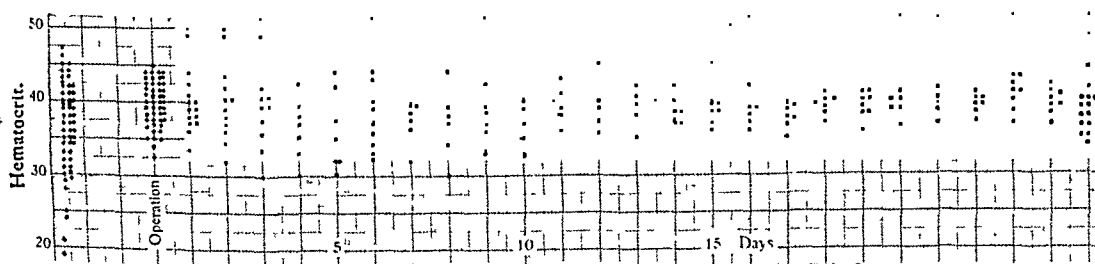
Fig. 5.

them has not had unusually great loss of protein. It is evident, therefore, that the regeneration of albumin in these patients is in an exceptional position, a fact which points in the direction of reduced albumin synthesis in the liver.

The globulins are on the whole normal or somewhat increased. The variation here is from 2.4 to 4.6 per cent (Fig. 3). The A/G quotient has been reduced in all patients. The increase of globulins by the preoperative treatment is relatively high, this quotient having rather decreased in the preoperative period. Thus, the pathological displacement within the fractions has been refractory to preoperative treatment. Contrary to that which was the case with the serum proteins it is found that the hemo-

globin values, which varied on admission between 48 and 118 per cent, relatively easily were brought to a fairly satisfactory level by ordinary preoperative treatment (Fig. 4). The values on the day of operation varied between 85 and 105 per cent. Hematocrit (Fig. 5) and the number of erythrocytes showed an analogous fluctuation.

In the postoperative phase a considerable fall takes place in the total proteins. The lowest values present themselves in the period from the 5th to the 10th day, when the average curve lies between 5 and 5½ per cent. After this period a slight rise occurs, though there is still a considerable hypoproteinemia at the termination of the first month, with average values below 6 per cent. These reduced values are found

not so rarely on control months or years after the operation, of course most frequently in the patients who show signs of recurrences. Also several patients, however, who are clinically symptom-free, do not infrequently demonstrate permanently reduced protein values.

Broadly, the albumin fraction shows the identical curve as the total proteins in the postoperativ period, with a more accentuated fall, however. This might also have been expected beforehand, as the preoperative concentration of this fraction proved more difficult to increase than was the case with the total proteins. Between the 5th and 10th day the values found lie between 2¼ and 2¾ per cent. In the subsequent postoperative course the increase is slight, so that there is still a large number of patients at the end of the first month in whom the values lie below the critical concentration for occurence of edema. Notwithstanding, it is relatively rare that these patients have had ankle edemas. The explanation of which may partly be that the administration of sodium and fluid in these patients as a rule has been somewhat on the low side compared to that usually given to operated patients.

The globulin fraction has demonstrated a lesser fall which takes place during the first few days of the postoperative period. Already from the 4th day the tendency in the concentration curve has been distinctly on the rise. During the entire course the A/G quotient as a rule has been less than 1. At the end of the first month the actual concentration most frequently has been lying between 3 and 3½' per cent.

The hemoglobin and hematocrit values have shown a quite negligible postoperative fall. After five days the values on an average have touched the immediate preoperative values, when no uncontrollable loss of blood has occurred in the postoperative period. In the subsequent course they have as a rule been within normal limits.

COMMENTS

Patients with high gastric carcinoma according to the present material demonstrate a considerable hypoproteinemia, which essentially depends upon reduction of the albumin fraction, as the globulin generally is slightly increased. A number of patients who on admission apparently have had normal total values, show a marked fall when they come into fluid balance, which may be ascribed to the dehydration. The degree of these changes is more pronounced than in patients with low-seated gastric carcinoma except those who have had symptoms of protracted pyloric stenosis or massive hemorrhage. There may be various causal relations of this.

Firstly, carcinoma in the cardiac region, particularly when it involves the oesophagus, causes considerable trouble for the intake of food before the patient enters hospital. When this is not the case these carcinomas have an insidious course, so that they may be of old date and may frequently show an enormous extent on admission. Many of them have to be included among those which until quite recently were regarded as inoperable. Through a much longer period than ordinary gastric carcinomas, therefore, they have been in a chronic state of negative nitrogen balance (Con Tui, 1949), frequently also anemia, which leads to considerable regressive changes in the liver (Efskind, 1949).

The postoperative reaction, as far as the serum proteins are concerned, are also far stronger than in ordinary subtotal gastrectomies. This may be explained partly by the fact that the operative procedure is considerably larger. This has a bearing only on the immediate postoperative reaction, however. The essential difference found in this material as against ordinary gastric carcinoma, is that it is difficult to eliminate the hypoproteinemia, and especially the hypoalbuminemia, and in many cases it represents a permanent condition. According to the few liver biopsies we have made in these patients during the operation, it appears as if pathological liver changes are greater than in ordinary gastric carcinoma.

Further, it is fact that many patients in whom total gastrectomy has been carried out, have difficulty in taking adequate nutrition perorally, so that they are hard put to it to fill their caloric requirement purely quantitatively. Even an intensive parenteral feeding does not appear to be able to keep them in nitrogen balance.

The excretion of nitrogen lies at a low level in urine as well as in faeces, patients with diarrhea excluded. The cause of their hypoproteinemia, therefore, does neither appear to depend on increased mobilization of protein nor decreased utilization of food protein.

The hemoglobin values and the number of erythrocytes demonstrate singularly slight reaction in the postoperative priod. As splenectomy has also been performed in most of the cases, these patients lack two organs which have a certain connexion with the following two conceptions, namely the stomach with the antipernicious principle and the splen as reserve supply of red cells,

regulator of the iron metabolism and of the blood volume. In the present material there has been no definite case of gastrectomy anemia. A small group of these patients, however, show a quite typical picture in the postoperative period. They have pain in association with meals, frequently accompanied by symptoms of regurgitation. As a result of this they have difficulty in obtaining the necessary fluid supply, as this does not reach higher than 1000 ml, not rarely even less. It is difficult to supply them perorally with more than 20—25 grms of protein, even if concentrated and hydrolysates are given. The total caloric value per day generally lies between 1000 and 1200. During this period there is a considerable loss of weight which can be stabilized between 80 and 100 pounds. The patients who get deficient food with regard to protein as well as to the caloric part, and in addition are dehydrated, generally demonstrate anemia. They show the clear picture of starvation cases apart from the lack of edema, which is due to the limited fluid intake. These cases, therefore, form an important pathophysiological and nutritive problem, which has to be taken into account when choice has to be made between total and partial gastrectomy, as the condition may be progressive and may end fatally. The pathogenesis, however, is sure to be more complicated than to be explainable on the basis of the protein metabolism alone. Thus, some of these patients show abnormal blood sugar curve by ordinary tolerance test. Some also show regular shock symptoms after intake of larger quantities of water, proving that a purely quantitative factor may also play a certain rôle.

SUMMARY

1. 50 patients with high gastric carcinoma, who have been treated by total gastrectomy, have been examined with a view to serum proteins and nitrogen balance in the preoperative and postoperative periods.

2. The majority of patients demonstrate considerable hypoproteinemia, which is dependent upon reduction of the albumin fraction, and is more pronounced than in patients with low-seated gastric carcinoma. It is difficult to eliminate by the enteral ·and parenteral nutritional substances and methods at disposal for the present.

3. Postoperatively there is a considerable fall in the albumin fraction, which reaches its lowest level on the 5th—10th days. The globulin fraction shows slight changes, as a rule with increased values.

4. In some of the patients these changes in the serum proteins are permanent to a certain extent. The causal relation is complicated, the most prominent part seems to be played by deficient nutrition. Histopathological liver changes together with the hypoalbuminemia speak for abnormal protein synthesis in the liver also playing an important part.

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STUDIES ON THE CIRCULATION OF BLOOD IN MAN
VI. THE PULMONARY CAPILLARY VENOUS PRESSURE PULSE IN MAN 1

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In 1946 Dexter et al. showed, that it is possible to obtain arterial blood from the pulmonary capillaries by means of a heart catheter, which is pushed sufficiently deep into the pulmonary artery to obstruct one of its small branches. This proves that no or insignificant arterial anastomoses exists between smaller branches of the obstructed artery and the not obstructed pulmonary branches in the neighbourhood. Thus the pressure in the obstructed capillary area should be directly transmitted to the fluid in the catheter and it should be possible to trace it with ordinary manometers. Mean pressure determinations from obstructed pulmonary arteries have recently been reported preliminary by Hellems et al. No tracings have been published. This and following studies aims to evaluate the significance of obtained curves and mean pulmonary capillary venous (pcv) pressure.

TECHNIQUE

The technique of venous catheterization with determination of cardiac output and simulthaneous recordings of blood pressures, the electrocardiogram, phonocardiogram and respiration has re-

cently been described. (Lagerlöf and Werkö 1949.) The pressure tracings were obtained with capacitive manometers according to Tybjaerg-Hansen and Warburg. Attached to intracardiac catheters the manometer system had a natural frequency of 20—30 cycles per sec. The damping of the manometer system was critical. In most cases it was further increased by the electrical filter III on the amplifier. The mean pressures were usually highly overdamped curves, obtained with filter IV on the amplifier. Catheters 9 F was preferred. In some cases they had to be exchanged against smaller catheters because of difficulties to introduce the catheter into the heart. After catheterization of usually the right stem of the pulmonary artery the catheter was pushed into the lung field under fluoroscopic control until resistance was felt. The patient was then asked to take a deep breath. It was then usually possible to push the catheter 3 to 5 cm further without much increased resistance. The tip of the catheter in most cases stuck about 2—3 cm from the diafragma cupol. Other locations were also used for the pressure tracings. When the catheter had reached its definite position, slight traction did not move the tip. In most cases, especially in those with elevated pressure, it was possible to obtain fully oxygenated blood samples from the catheter.

In a few cases the blood in the first attempt was darker than the arterial blood. The catheter was then withdrawn and placed in another branch of the pulmonary artery and the procedure repeated.

After the sampling of blood, the catheter was flushed with saline and connected to the continuous
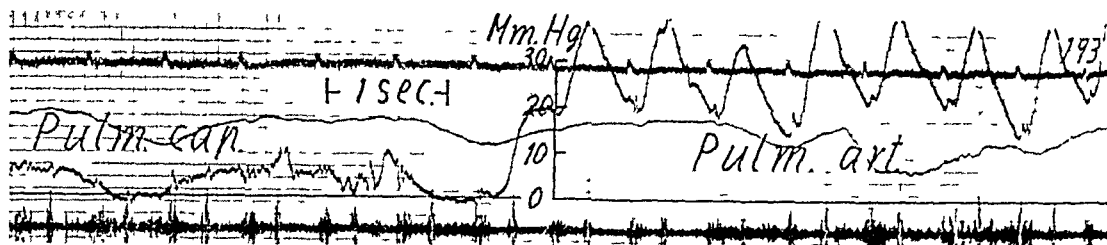
Fig. 1. Withdrawing the catheter from an obstructed branch of the pulmonary artery to the main stem in a case of auricular septal defect Overdamping by means of paraffine oil in the capacity. Undesigned line here as in all other curves respiration, inspiration downward, expiration upward.
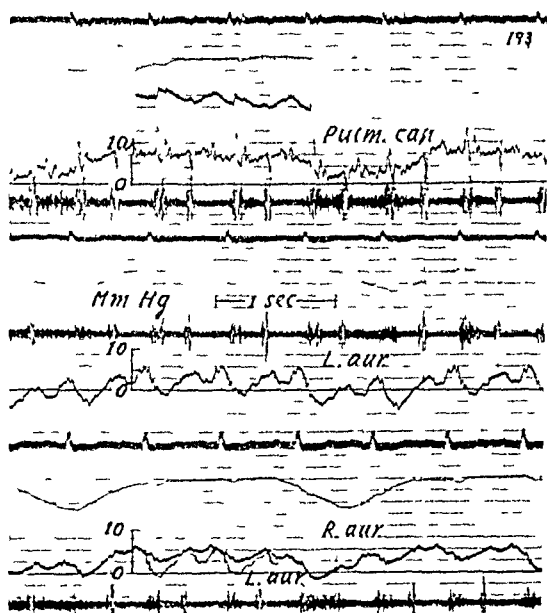
Fig. 2 Electrically slightly overdamped curves from the same case as in Fig 1
Systolic murmur.

drip. There was no resistance to the flow The catheter could without harm be left in the obtained position for at least ten minutes It was however usually connected to the manometer as soon as possible and the pressure pulse traced The catheter was then withdrawn to the pulmonary artery under continuous tracing The change from a typical venous pulse to an arterial pulse with higher mean pressure could be read immediately on the amperemeter of the amplifier or on the light spots of the oscillographic mirror It

could be used in repeated tracings to place the tip of the catheter without fluoroscopic control

Altogether 50 patients have been investigated, normals, pregnant women, cases of hypertension, mitral valve disease and pulmonary emphysema Examples of representative curves are given below.

In most cases venous curves were obtained with markedly lower mean pressure than in the pulmonary artery and amplitudes equal to or larger than those of the right auricle. In some cases with a low pcv mean pressure the amplitudes were small especially during expiration, probably because of damping in the pulmonary capillaries and arterioles. Respiratory variations of pressure always appeared

In three cases with large hearts it was impossible to reach the thinner arteries because of insufficient length of the catheter In one case with a history of pulmonary emboli the catheter met resistances which did not allow passage into a sufficient small artery.

COMPARISON BETWEEN LEFT AURICLE AND PCV PRESSURE

Pcv and left auricle pressures were obtained in one 50 years old woman with auricular septal defect without signs of decompensation. Clinical data and other findings at heart catheterization are found in Table I. The catheter was first placed in the pulmonary capillaries and then withdrawn to the pulmonary artery, right ventricle and auricle. After some manipul-

Table 1. Systemic flow 3.0 l/min. Pulmonary flow 10.0 l/min.

Case	Heart size ml/m² BSA	Oxygen content, ml/l				Oxygen capacity	Blood Pressures, mm Hg					
		Brach art	Pulm art	Rt aur	L aur		Brach art	Pulm art	Rt ven	Rt aur	Pcv	L aur
193	390	20.3	17.8	17.3	20.1	22.9	120/80	40/18	40/6	3	3.0	2

ations it was then placed in the left auricle and withdrawn to the right. The pcv and left auricular tracings were done within half an hour, the left and right auricular tracings within two minutes. The pulse time varied less than 0.02 seconds in the different tracings indicating a relatively unchanged basal state.

Fig. 1 demonstrates the sudden rise of pressure and change of configuration of the curve when the catheter suddenly loosens from the obstructed small branch of the right pulmonary artery. The curve is overdamped by means of paraffine oil in the capacity. Fig. 2 illustrates the form of the pcv, left and right auricular pressure pulses, obtained with slight electrical overdamping. The venous caracter of the pcv tracings is masked by artefacts but is easily seen on a superimposed higher damped curve, whose amplitudes are magnified twice. Its pressure summits are about 0.09 seconds delayed as compared with the corresponding in the left auricle. The different phases of the left and right auricular pressure tracings are the same as have already been described (Lagerlöf and Werkö 1948). The amplitudes of the left auricular tracing are greater than in the right. In absolute figures they do not exceed what is often found in the right auricle. The pressure during systole and early diastole are lower than in the right, indicating a right to left shunt during these periods. Such a shunt was further eviden-
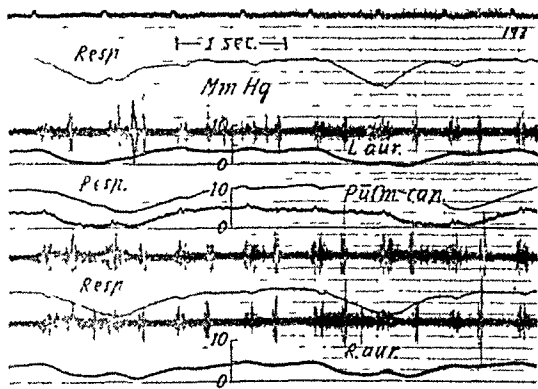
Fig. 3. Electrically overdamped curves from the same case as in Fig. 2. Pcv mean pressure 2 mm Hg higher than the left auricular.

ced by an only 90 per cent oxygen saturation of the left auricular blood. It is probable that the balance of pressure between the two auricles rapidly changed and that the mean pressure of the left was usually higher than in the right because the determined pulmonary flow was three times greater than the systemic.

Fig. 3 shows electrically highly overdamped tracings of left auricle, pcv and right auricular pressure. The mean pressures are 2, 3 and 3 mm of mercury.

THE NORMAL CAPILLARY VENOUS PULSE

Fig. 4 shows the pcv pulse in a case of hypertension without decompensation and with normal pressures in the lesser circulation.

Right auricular and pulmonary artery pressure tracings with the same pulse times and
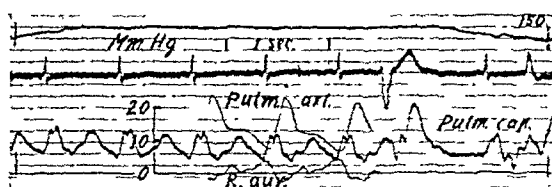
Fig. 4. Pcv pressure tracing in a case of compensated hypertension. Note the ventricular extrasystoles.
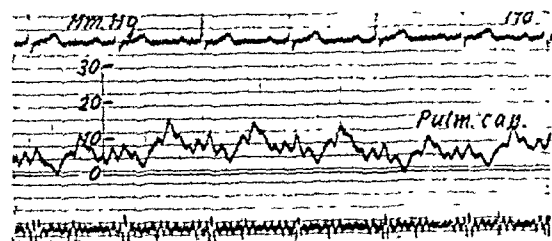
Fig. 5. Pcv pressure tracing in a normal young man.

in the same phase of respiration are superimposed with the aid of the ecg. The pulmonary artery pressure tracing is smoothed. The pcv pressure pulse has no similarity to the pulmonary artery pulse. It has the following features common to the right auricular pressure pulse: 1) A positive auricular wave. 2) An early systolic dip of pressure followed by a late systolic and early diastolic increase of pressure. 3) A middiastolic decrease of pressure. The essential differences between the pcv and right auricular pressur are: 1) A considerably higher mean pressure. 2) A delay in time of about 0.08 seconds. 3) A prolonged diastolic pressure fall which continues until the auricular pressure wave appears. During this time the pressure fall parallells that of the pulmonary artery. The higher mean pressure and the delay in times are found in all, the prolonged diastolic pressure fall in most pcv pressure tracings.
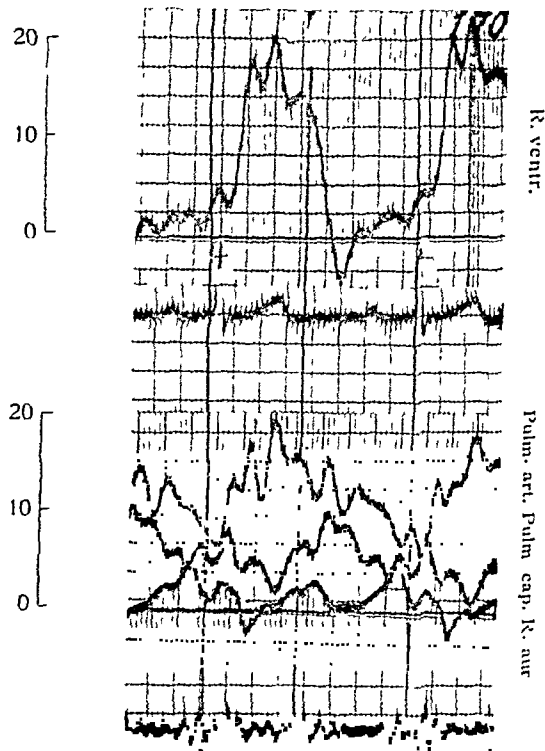
Fig. 6. Superimposed curves from the same case as in Fig. 5.

Fig. 5 shows the pcv pulse from a helthy young man, fig. 6 superimposed curved from the same case. It shows essentially the same as Fig. 4 but less clear because of oscillations which probably are artefacts.

VENTRICULAR EXTRASYSTOLES

The last beats in Fig. 4 illustrates the effect of ventricular extrasystoles on the pcv tracing. The start of the auricular contration may be judged approximately from the ecg, assuming that it comes in normal times after the preceeding p-wave. The auricular pressure wave here appears almost instantaneously after the auricular contraction, probably because the pulmonary veins

are filled with blood when the left auricle contracts and the elasticity coefficient and the pulse wave velocity is high. As in the right auricle under similar conditions the obtained pressure is much higher than in normal beats (Bloomfield et al. 1946, Lagerlof och Werkö 1948). During the compensatory pause the pressure is almost constant for about 0.3 seconds.

Other ventricular extrasystoles are seen in Fig. 8.

AURICULAR FIBRILLATION

Fig. 7. demonstrates the pcv pulse in a case of grave hypertension with acute left ventricular failure and transient rapid auricular fibrillation. Clinically the patients was on the limit of pulmonary edema with dyspne, orthopne and moist and dry rales on the bases of the lungs. The mean pcv pressure was about 30 mm of mercury, i.e. higher than the colloid osmotic pressure of the blood.' The right auricular pressure was normal. A marked third sound was heard on the apex.

The respiratory variations are great and amounts to about 15 mm of mercury. The highest pressure is reached in the middle of expiration, the lowest in the middle of inspiration.

Synchronous with the first sound a deep pressure dip is seen, probably an artefact. The pressure then rises sharply during few hundreds of a second, evidently because the pulmonary veins rapidly fills from the distended pulmonary artery when the mitral valve is closed. The pressure rise suddenly stops about 0.06 seconds after the begin of the first sound and levels off for another 0.06 seconds probably as a combined result first of the descens of the base and second

of the equalization of pulmonary artery and venous pressure. The subsequent course is different in different beats. In the beats which are preceeded by beats with a pulse time of more than 0.32 seconds a great late systolic and early diastolic pressure rise is seen, which begins some hundreds of a second prior to the second sound and reaches a maximum in the middle between the second and third sound. In the beats which are preceeded by beats with shorter pulse time this pressure rise is lacking. Whether or not this late systolic and early diastolic pressure rise appears the pressure falls sharply about 0.03 second before the third sound which in short beats confluences with the first sound. No auricular pressure wave is seen.

The relation between the late systolic and early diastolic pressure rise and the duration of the preceeding beat are explained by the pulse deficit in the pulmonary artery seen in other tracings from the same patient. If no systolic pressure rise occurs in the pulmonary artery the pressure difference between the pulmonay artery and pulmonary veins will keep constant until the mitral valve opens.
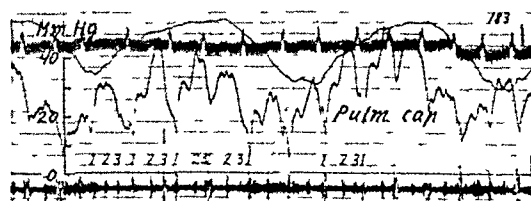
Fig. 7 Pcv pressure pulse in a case of malignant hypertension on the limit of pulmonary edema Note the high mean pressure and the great amplitudes, the influence of the duration of the foregoing beat on the shape of the wave, further the high amplitude of the third sound, when the early diastolic pressure is unusually high (at the arrow).
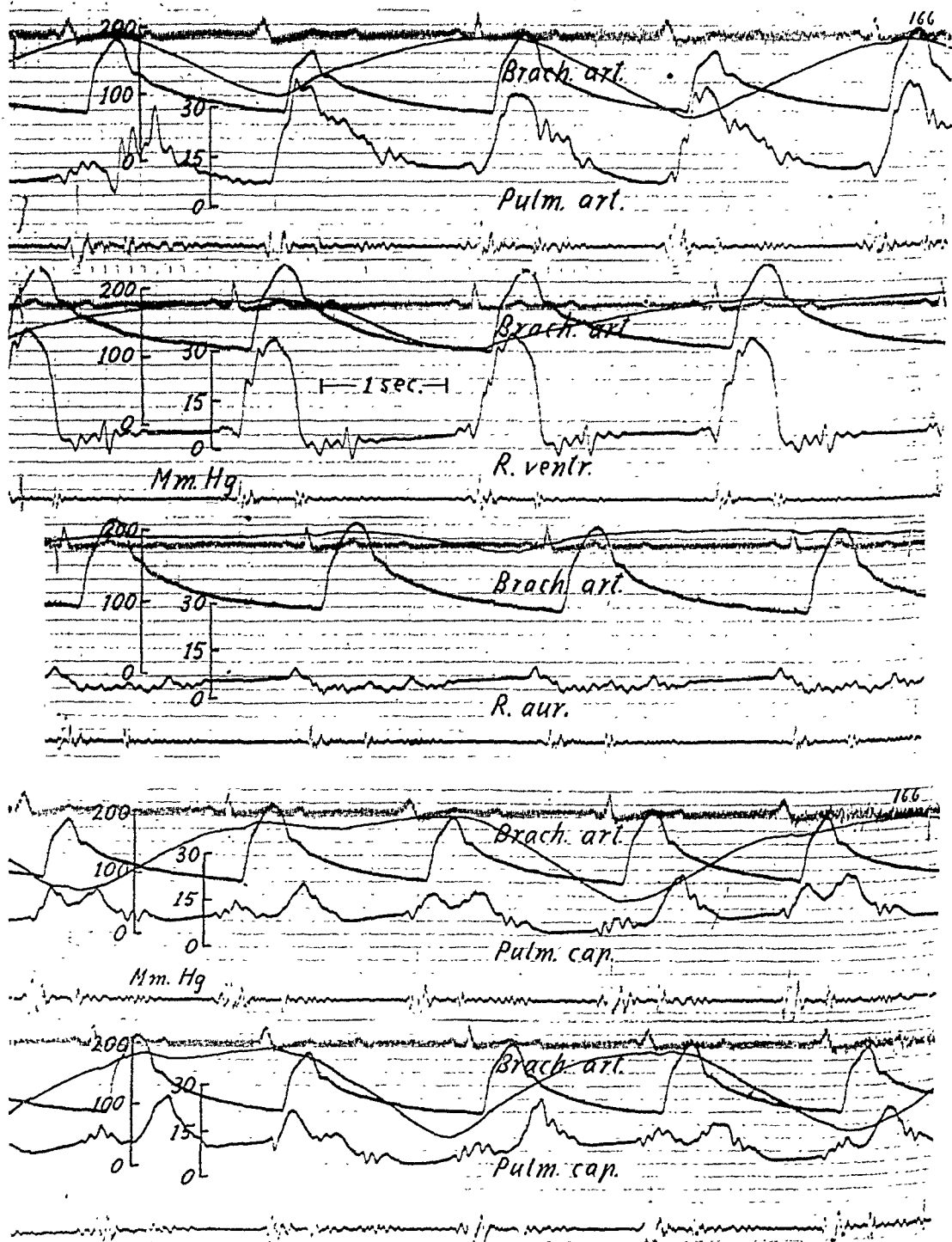
Fig. 8. 1—2 block with ventricular extrasystoles. Note the different amplitudes of the auricular waves falling in different phases of the cardiac cycle. Low frequency auricular sounds.

A—V BLOCK

Fig. 8 shows tracings from a 60 years old woman with hypertensive cardiovascular disease and a 1—2 A—V block and ventricular extrasystoles.

In the beats where the excitement of the ventricles in a normal way are preceded by auricular contraction, the pulmonary capillary venous pulse is similar to that already described in normal cases. The sharp decrease of pressure early in diastole which is supposed to be the delayed effect of the opening of the miral valve here appears 0.15 to 0.20 seconds after the begin of the second sound. Long duration of the isometric relaxation in the left ventricle is to be expected in this case with hypertension and very slow pulse rate.

The blocked auricular contraction which appears during the phase of rapid filling, similar to right auricular contraction during the same phase, does not cause any evident pressure rise in the pcv pressure.

The decrease of pressure after the opening of the mitral valve continues for about 0.35 seconds parallell with a similar decrease of the pulmonary artery pressure. After this time the pressure is first constant for about 0.15 seconds and then rises a few mm of mercury during the following 0.4 seconds. During this period the pulmonary artery and the pcv pressures are almost equalized.

The effect of auricular contractions, which appear just before or during ventricular contractions are seen in the ventricular extrasystoles. It is similar to the effect on the right auricular pressure described earlier (Lagerlöf and Werkö 1948)

Fig. 9. Pcv pressure with superimposed pulmonary artery and right auricular pressures in a case of mitral stenosis regurgitation without decompensation.

MITRAL STENOSIS AND REGURGITATION

Fig. 9 shows tracings from a 54 years old female with a history of rheumatic fever 14 years ago, moderately enlarged heart (550 m/m² body surface area) and backward bulging of the left auricle. A loud presystolic murmur and an opening snap was heard on the apex. No systolic or middiastolic murmurs appeared. Ecg, respiration, pcv pres-
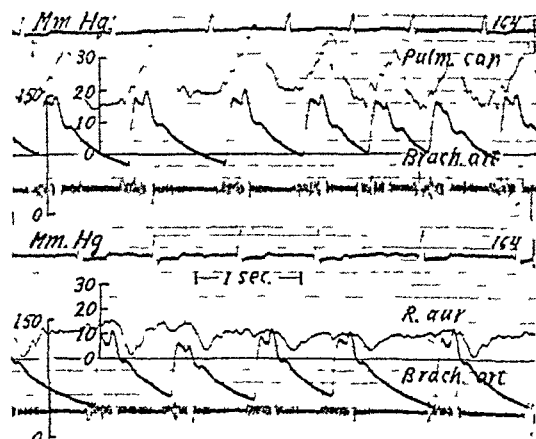
Fig 10 On the top: Pcv and brachial artery pressure in a case of rheumatic mitral stenosis and regurgitation in failure. Note the strong systolic and faint diastolic murmur, further the absence of any auricular pressure wave. Below. right auricular and brachial artery pressure in the same case. Note the absence of systolic dip in the auricular curve.

Fig. 11. Superimposed curves from the same case
as in Fig. 10.

sure wave and 0.03 seconds before the pre-systolic murmur. The diastolic pressure fall begins 0.14 seconds after the second sound and 0.03 seconds after the opening snap.

Fig. 10 illustrates electrocardiogram, pcv pressure, brachial artery pressure and phono-cardiogram for middle high frequences in a 30 years old man with a rheumatic mitral stenosis and regurgitation, tricuspid regurgitation and auricular fibrillation with decompensation. The next figure (11) shows superimposed curves from the same patient The strong systolic and faint middiastolic murmurs in the fifth intercostal area are easily seen in the phonocardiogram.

The mean pressure is high, amounting to 24 mm. The respiratory variations of the pcv pressure are of the same magnitude as in the first cases. There is no sign of auricular activity. The pcv pressure begins to rise gradually about 0.4 seconds after the begin of the first sound almost simultaneously with the pulmonary artery pressure and in contradistinction to normal continues to rise during the whole systole. It reaches its summit 0.10 seconds after the begin of the second sound and then falls rapidly during the following 0.20 seconds. The pressure fall is deeper than in the pulmonary artery during this period of time. In beats with short diastoles the pressure fall is immediately followed by the systolic pressure rise of the next beat, in beats with long diastoles a period follows with almost constant pressure, which only increases or decreases little with the phase of respiration indicating a balance between inflow from the still distended pulmonary artery and outflow through the stenosed valve. During such periods the diastolic murmur is less marked indicating decreased A—V flow.

sure and phonocardiogram were taken simultaneously, right auricular and smoothed pulmonary artery pressures have been superimposed.

The pcv mean pressure and its mean features are essentially the same as in the normal tracing. The auricular wave is however relatively more marked. It appears 0.09 seconds after the right auricular pres-
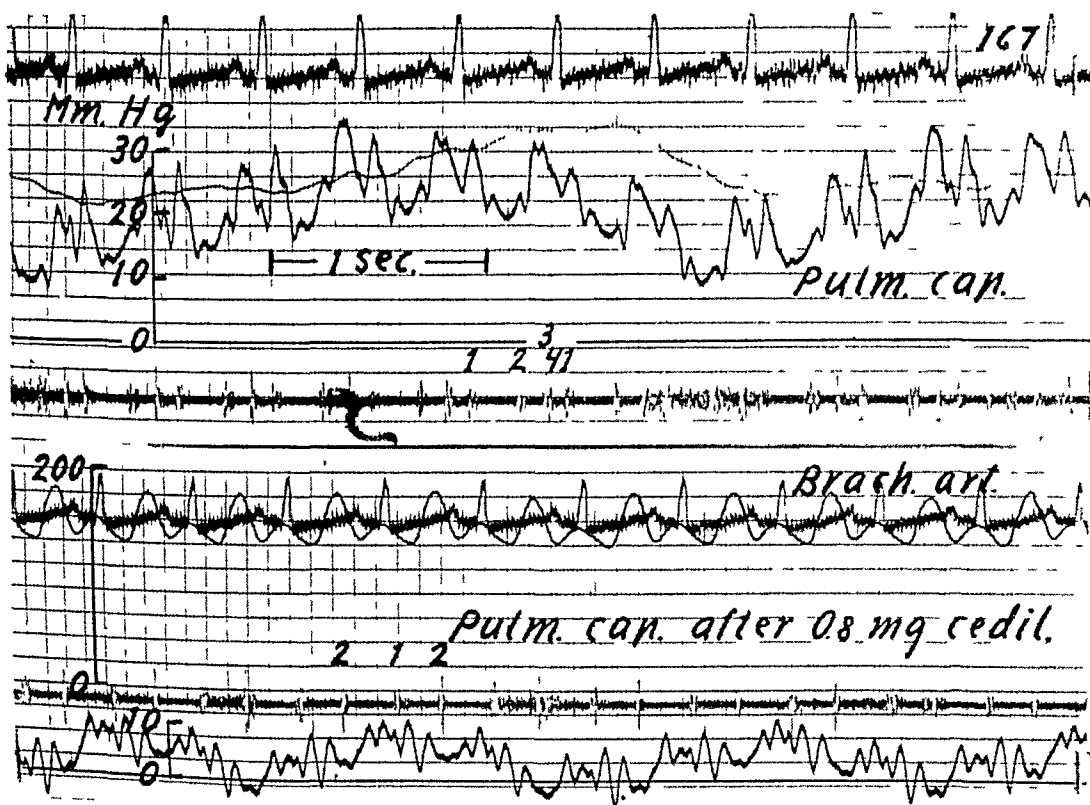
Fig 12   On the top. Pcv pressure in a case of hypertension on the limit of pulmonary edema
Below pcv and brachial artery pressure 40 min after 0 8 mg cedilamide i v. Note the disappearance
of gallop rhythm when the pcv pressure decreases

We interprete the immediate high systolic pressure rise without systolic dip as evidence of the mitral regurgitation, indicated by the systolic murmur   The difference in shape between the right auricular pressure pulse in tricuspid regurgitation and the pcv pulse in mitral regurgitation is marked

GALLOP RHYTHM

It is generally held (for literature see Mannheimer), that the third and fourth heart sound mainly are the result of the vibrations in the ventricular walls, caused by the rapid inrush of blood from the auricles and that an audible gallop only is due to exaggerations or confluence of normal sounds   A prio reasoning has led to the conclusion, that a greater pressure difference than usual between the left auricle and ventricle should be one of the causes of gallop rythm but experimental evidences are lacking.

Two of our patients had a pronounced gallop rythm. The main clinical datas and the pcv pressure tracings of the first case are already given on page 5 and in Fig. 7. The presence of a third sound was bound to the length of the diastole. When the

early diastolic pressure fall coincided with the isometric contraction and insignificant amounts of blood reached the left ventricle as evidenced by the appearance of the tracings of the next beat, then no third sound appeared. In the other beats a third sound appeared about 0.04 seconds after the begin of the steep diastolic pressure fall. In one beat where the early diastolic pcv pressure rised to 45 mm of mercury and the steep pressure fall amounted to 28 mm of mercury the third sound had the same amplitude as the first sound. In others where the early diastolic pressure and pressure fall were less marked the third sound had a much lower amplitude.

The second case had hypertension with severe signs of left ventricular failure and a rapid normal rhythm.

The pcv tracings are found in Fig. 12. The mean pressure was 20 mm Hg. The early diastolic pressure rised to 30 mm of mercury. The sharp decrease of pressure started 0.12 seconds after the second sound. It did not reach the systolic level before the auricular wave started, i.e. the auricular contraction coincided with the last part of the rapid filling of the left ventricle. The loud third sound appeared 0.02 seconds after the early diastolic pressure fall at about the time the auricular wave had reached the pulmonary veins. There was evidently a summation gallop.

40 minutes after the injection of 0.8 mg of cedilanide the pcv mean pressure fell to 3 mm of mercury and the amplitudes of the diastolic pressure changes had decreased markedly without any marked changes of heart rate. The third sound had disappeared.

DISCUSSION

If no capillary anastomoses exist between the obstructed capillary region and the adjacent regions the former may be regarded as a part of the manometer system. The traced pressure will be the pressure in the part of the venous system, where the veins from the obstructed area converge with those from the not obstructed. The total manometer system will have a lower frequency than the original, because of the low elasticity modul $\dfrac{\Delta P}{\Delta V}$ of the capillaries. The natural frequency is not constant as the elasticity is higher at high pressure than at low. Thus coupled oscillations may occur. The damping of the system will probably partly take place in the pulmonary arterioles. The amplitudes of high frequences will as in all manometer systems decrease most.

From the statements above it is clear, that the pcv pressure curve reflects only the lowest frequences of the pulmonary venous pressure, i.e. its main features. Further the pressure differences in the pulmonary veins are traced with a considerable time lag. The delay in time is greater the less the pressure at the moment is.

In the pcv pressure tracings as in other catheter tracings oscillations of a frequency of about 10 per second are often seen, especially at the end of auricular systole, begin of ventricular systole and at the begin of diastole. These oscillations which are most pronounced in cases with rapid heart rate, most certainly are artefacts caused by catheter motion, due to rapid blood flow or impact of auricular or ventricular structures including the valves. They appear without any time lag often syncronous with oscilla-

tions in the phonocardiogram. To a certain degree they may be used to time different cardiac events. In the analysis of the pressure tracing these oscillations must be excluded by arithmetical smoothing or, as in most of our cases, by a certain degree of overdamping. The curves obtained in this way have the smooth appearance as any tracing with a manometer of relatively low frequency. They do not give a true picture of rapid variations of the pulmonary venous pressure and usually do not allow a distinct timing of its different phases. They can, however, be used to measure the mean pressure, and further allow approximative statements about time relation and amplitudes.

The mean pressure in the pulmonary veins should be slightly higher than in the left auricle, because some pressure energy is lost by friction. Observations of Cournand et al. (1947) in three cases of auricular septal defects indicates that the pressure difference amounts to about 2 mm of mercury. This pressure difference is equal to that found by us between pcv and left auricular pressure in our case of auricular septal defect. Hellems et al. (1948) in one similar case found no significant difference between pcv and pulmonary venous pressure. Thus the pulmonary venous pressure can be measured by means of pcv tracings.

Cournand et al. (1947) in three cases and Lagerlöf and Werkö (1948) in two cases of auricular septal defects found the left auricular mean pressure to be some mm of mercury higher than the right. In the same direction speaks the clinical experience, that in auricular septal defects the blood is usually shunted from left to right. The case reported here showed a somewhat higher mean pressure in the right than in the left auricle. This was probably partly due to small changes in pressures between the separate determinations. A small degree of oxygen unsaturation (10 %) indicated a shunt from the right to the left whereas a three times greater pulmonary than periferal blood flow indicated a great shunt from left to right.

The generally higher pressure in the left auricle in cases with auricular septal defects indicates, that normally the left auricular mean pressure is higher than the right (Cournand et al. 1947). It must be assumed, that a great left to right shunt decreases the left auricular pressure, and increases the right. Increased right ventricular filling pressure is also a presumption for the increased pulmonary flow if the pulmonary resistance is normal or as in our case increased. Conclusive evidences of a higher mean pressure in the left auricle are given by the pcv mean pressures obtained by us which in all cases except three were 4 mm or more higher than the right auricular.

The pulmonary resistance in man has hitherto been calculated from the differences between the pressures in the right auricle instead of the left and the pulmonary artery or from the p. a. pressure alone. In this way too high figures have been obtained especially in left ventricular failure. Correct figures are obtained when the right auricular pressure is exchanged to the pcv mean pressure less 2 mm of mercury.

The mean pcv pressure in half recumbent position referred to a level of 5 cm below the angle of Louis in three decompensated cases have been presented in Figures 7, 10, and 12. It amounted to 30, 24, and 20 mm

Hg. Because of less frictional losses and less linear velocity the capillary pressure is higher. Because of addition of a hydrostatic component the capillary pressure in the lung bases is further about 15 mm, higher, i. e. considerably higher than the colloid osmotic pressure of the blood. This is the first demonstration that the pulmonary capillary pressure may rise sufficiently to cause a filtration of fluid. The presence of fluid in the basal pulmonary alveoli and fine bronchs was evidenced by abundant small rales. Frank pulmonary edema developed, when a recumbent position was maintained for some minutes.

If the left auricle contracts when the mitral valve is normal and open and the pressure is low the resulting pressure wave in the pcv tracing usually has a low amplitude of one or few mm of mercury. When it contracts against a stenosed or closed valve or the pressure is high the amplitude may be much higher as shown in Fig. 9. The auricular cause of this pressure waves is born out by the facts that it starts before the Q-wave in the electrocardiogram, that it is not seen as a result of a normal heart beat in auricular fibrillation and that it is delayed in extrasystoles. Assuming, that the left auricle contracts 0.02 seconds after the right auricle we may calculate the time lag between the left auricular contraction and the traced pressure. It amounts with considerable variation to about 0.04 seconds. During the isometric contraction of the ventricles the pcv pressure continues to rise for about 0.4 seconds. The rise is probably mainly due to the delayed auricular pressure wave and only to a small degree to bulging of the mitral valve and arrest of the blood flow through the mitral valve. In 3 cases

with auricular fibrillation and in one case with extrasystoles the early systolic increase was insignificant. The magnitude of the systolic dip is mainly governed by the height of the auricular wave. The pressure seldom decreases markedly below the values before the auricular wave. In contrast to the conditions in the auricles and in the periferal veins the pressure and filling of the pulmonary veins is thus about the same during systole and diastole before auricular contraction. This is explained by the great systolic flow through the pulmonary capillaries evidenced below and the distance to the left ventricle, which allows a considerable amount of blood to enter the pulmonary veins before or simultaneous with the negative pressure wave of the left auricle. The dip reaches its minimum in the middle of systole and from then on the pressure climbes to a maximum about 0.12 seconds after the second sound. At this time the pressure falls distinctly during one or two tenth of a second, evidently because of emptying of the veins through the left auricle to the left ventricle. Three cases with opening snap of the mitral valve showed a rapid pressure fall about 0.03 seconds after the opening of the mitral valve. The diastolic pressure fa" is usually more marked than the corresponding phase in the right auricle and systemic veins. The pressure fall in long diastoles is usually followed by a period of almost constant or rising pressure indicating balance between inflow and outflow. In short diastoles it is immediately followed by the auricular pressure wave.

The amplitudes of the different waves differ much in different cases evidently at least partly due to different degree of damping. They are greater the higher the mean pressure
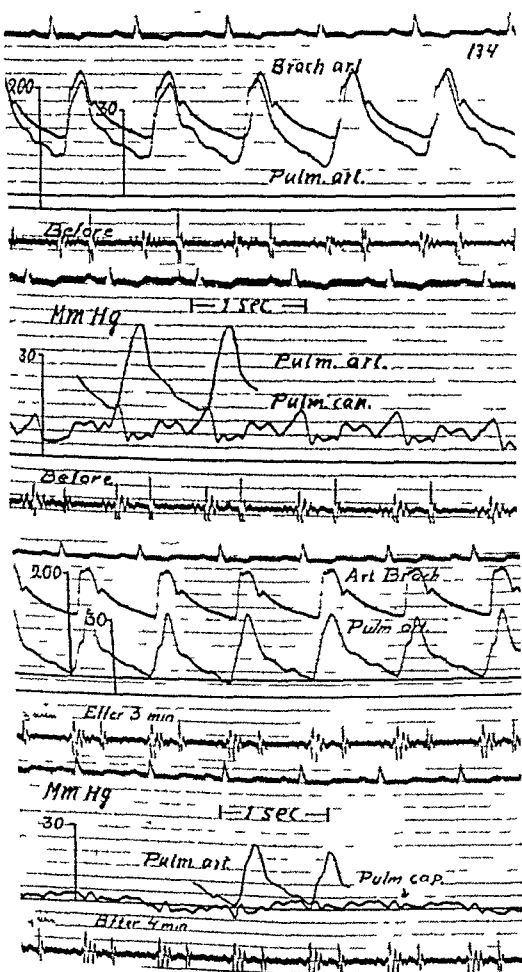
Fig 13   Pulmonary artery   Brachial artery and pcv pressures in a case of hypertension before and 4 min after 0 4 g theophylline-diaethanolamine i v   Note the decrease of the pcv pressure

sure is   This is shown in Fig 12 and 13, where the mean pressures fell considerably after the administration of 0 4 g theophyllin-diethanolamine or 0 8 mg lanatoside c intravenously   In good curves they are usually greater than in the right auricular curves from the same patient.   This is to be expected because of the higher mean pres-

sure and elasticity coefficient and further bcause of the smaller capacity of the pulmonary veins than of the periferal veins (Cournand et al 1947).   The per cent volume change of the system caused by opening of the A—V valves therefore will be much greater in the pulmonary veins.   In contrast to the conditions in the right auricle, the highest pressure in cases with a normal mitral valve usually appears at the time the A—V valves open.   The lowest pressure is more often found in the middle or end of diastole than during systole.

Stenosis and incompetency of the mitral valve may change the appearance of the curve   In stenosis the energy at the auricular contraction is not in a normal manner converted into energy of motion but into a backward reflected pressure wave.   The auricular pressure wave is therefore higher than normal.   In incompetency the auricles and pulmonary veins are filled during systole simultaneously from the pulmonary artery and the left ventricle   The result is an unusually early and high systolic pressure rise The difference in appearence between the pcv pressure pulse in mitral regurgitation and the right auricular pressure pulse in tricuspid regurgitation (Fig. 10) is explained first by the higher amplitudes and second by the low resistance of the pulmonary arterioles, allowing a rapid equalization between the pressures in the pulmonary arteries and veins   The pcv pressure pulse adds to the murmurs in detecting mitral lesions, further to judge the relative hemodynamic importance of the stenosis or the incompetency of a decreased mitral valve, or to diagnose a relative incompetency of the same valve.

Our pcv tracings represent, as discussed before, a somewhat distorted and few hundreds of a second delayed pressure pulse of the small pulmonary veins. The pressure in the small arteries situated some centimeters from this veins does, as special experiments show, not differ from that in the main stem of the pulmonary artery with exception for a time lag of about 0.03 hundreds of a second. By superimposing the pcv and pulmonary artery pressures in the same period of respiration with regard payed to the time lag it is possible to get an approximative idea of the pressure difference between the small pulmonary arteries and veins.

mm Hg
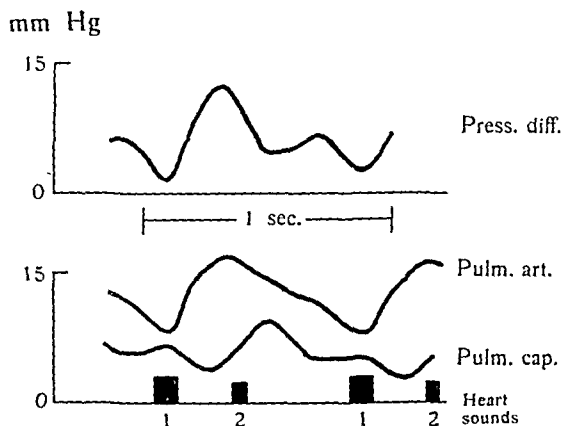
Fig. 14. Pressure differences between the small pulmonary arteries and veins in a normal case (the same as in Fig. 5).

Fig. 14 gives the pressure differences obtained in the normal case in Fig. 5. During the isometric contraction of the ventricles the pressure difference is small. It increases rapidly during ejection phase and reaches its maximum in the last third of the systole. From here on it decreases until the mitral valve opens, and then rises a little again until the left auricle contracts and then decreases again. During the isometric con-

traction it reaches its lowest value. Other similiar constructions all show the summit of the pressure difference to be late systolic. The lowest values are sometimes found at the end of the isometric relaxation of the ventricles sometimes as in this case during the isometric contraction.

If we assume the resistance in the arterioles and capillaries to be constant and the velocity of the blood flowing through the small arteries to be small the flow through the capillaries is approximately proportional to the pressure difference. If the pulmonary resistance should change during the cardiac cycle it should decrease during systole when the arterioles are distended. If the velocity of the blood in the pulmonary arteries plays a role for the capillary flow this should increase during systole. All these facts indicate that the pulmonary capillary flow is greatest during systole.

SUMMARY AND CONCLUSIONS

1. The pressure in small branches of the pulmonary artery obstructed by a heart catheter has been recorded. The obtained pulse is called the pulmonary capillary venous (pcv) pressure pulse.
2. Resistance to the catheter in the pulmonary artery before sufficient small branches are reached indicates earlier pulmonary emboli.
3. The mean pcv pressure equals the mean pulmonary venous pressure. It is about 2 mm of mercury higher than the left auricular mean pressure.
4. The left auricular mean pressure in most cases is higher than the right.
5. The pcv mean pressure and hence the capillary pressure in left ventricular

failure may be elevated above the colloid osmotic pressure of the plasma.

6. The pcv mean pressure may be decreased by intravenous injection of 0.4 g theophyllin-diethanolamine or 0.8 mg cedilanide.

7. The pcv pressure pulse in different conditions shows the same main phases as the right auricular but delayed. The time relations to the ecg and the cardiac sounds and murmurs are exemplified and discussed.

8. The shape of the pcv pressure pulse may be of importance in evaluating the function of the mitral valve.

9. The import of the increased pulmonary venous pressure in gallop rythm is demonstrated.

0. The pressure difference between the pulmonary arteries and veins are greatest during systole. This indicates that the systolic flow through the pulmonary capillaries is greater than the diastolic .

11. The pulmonary vascular resistance can be calculated from the pressure difference between the pulmonary arterial and the pcv pressure minus 2 mm of mercury.

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·A NEW, PREVIOUSLY UNKNOWN CLOTTING FACTOR

By investigation of a case of hemorrhagic diathesis in 1943 it was shown that the condition was caused by the lack of a previously unknown clotting factor, which was termed the fifth clotting factor, or factor V (Owren 1944). Under physiological conditions this factor is necessary for the conversion of prothrombin to thrombin. It was further shown that factor V is an inactive substance, which, during the coagulation process, is transformed into the active form, termed factor VI. Factor VI plays a fundamental rôle in the conversion of prothrombin to thrombin. The conversion rate increases with increasing amounts of factor VI (Owren 1947).

It was previously assumed that factor VI together with calcium converts prothrombin to thrombin. It has now been shown by experimental investigations that this is not the case. Even by adding thromboplastin (human brain extract) to a mixture of prothrombin, factor VI and calcium, the thrombin formation is slow and incomplete. If, in addition, a small amount of normal plasma is added, the thrombin formation proceeds rapidly. By preparation and purification experiments it has been shown that the factor in normal plasma which exerts this effect is of a protein nature, and it is not identical with any of the previously known clotting factors since it has been prepared free from prothrombin, factor V, antihemophilic globulin and fibrinogen. This substance therefore represents a new clotting factor. It is a component of he activating complex which converts prothrombin to thrombin.

Contrary to what has previously been stated, among others by Quick (1947), we find that the greater part of the prothrombin is present in the serum several days after spontaneous coagulation of normal blood. Only a small proportion of the prothrombin is transformed into thrombin during coagulation. The rest of the prothrombin is found in the serum, and this part is slowly inactivated in the course of several weeks. Quick's so called prothrombin consumption test (Quick, 1947) has therefore nothing to do with loss of prothrombin during coagulation. When Quick finds that the prothrombin has disappeared 1—3 hours after spontaneous clotting this is caused by the fact that the prothrombin in the serum after this period, can no longer be detected by his method. The new factor rapidly disappears from the serum after coagulation. This is the reason why prothrombin in the serum is not transformed to thrombin and it is partly the lack of this new factor which is responsible for the erroneous results of Quick's prothrombin test in serum. The fibrinogen, which is added in performing this test, does not contain the new factor. Warner, Brinkhous, and Smith also found (1936) that prothrombin disappeared in serum after spontaneous clotting. This is caused by the fact that the prothrombin in serum is not detectable by the original two-stage method,

since the prothrombin in serum is not converted by the addition of thromboplastin alone. The previously described one-stage method for prothrombin assay (Owren 1947, 1949) allows, however, determination of the prothrombin in serum, since the prothrombin-free ox plasma, which is used, contains the new factor. The same is the case with the two-stage method described by Owren (1947), as is has been found that the factor V-preparation which is used in the conversion mixture, and which is prepared by iso-electric precipitation from prothrombin-free ox plasma, contains both factor V as well as the new factor.

Further investigations regarding the preparation and properties of this new factor will be published later.

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   July 1949.

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Reviews

PORPHYRINS AND THEIR DETERMINATION. A REVIEW[1]

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By T. K. WITH

Porphyrins are *cyclic tetrapyrroles* derived from the compound porphin which consists of four pyrrole rings attached to eachother by four methene bridges. Porphin was originally proposed (16) as a hypothetic basic skeleton but was later synthesized (15, 54). Two isomer forms are possible which show mesomeria (11).

Fischer speaks of "dualism of porphyrins". Because they cannot be transformed into each other without opening of the porphyrin ring, re-arrangement of its pyrrole rings, and formation of a new ring, I and III must be synthesized independently.

Starting from the etioporphyrins — which do not occur in nature — the *coprapor-*
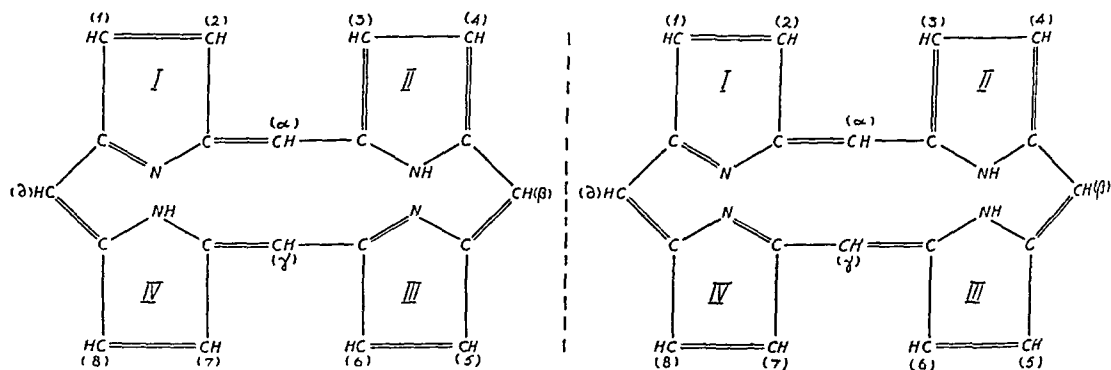
Fig. 1. The two mesomeric forms of porphin. The pyrole rings I—IV, the methene bridges α—∂, and the H-atoms 1—8 are seen.

The formulae of the porphyrins are most conveniently deduced from the four *etioporphyrins* (tetramethyl-tetraethyl-porphins) named I—IV. Only compounds derived from I and III do, however, occur in nature, and as both are found in animal products

phyrins (tetramethyl-tetra propionic acid-porphins) are derived by carboxylation of the four ethyl groups. If the methyl groups are carboxylated too one obtains the *uroporphyrins* (tetra acetic acid-tetra propionic acid-porphins).

If two of the ethyl groups are unchanged and the other two carboxylated, one comes to tetramethyl-diethyl-dipropionic acid-por-

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[1] In slightly modified form read at "Tredie Nordiska Laboratorieläkaremotet", Ed. Sweden, June 1948.

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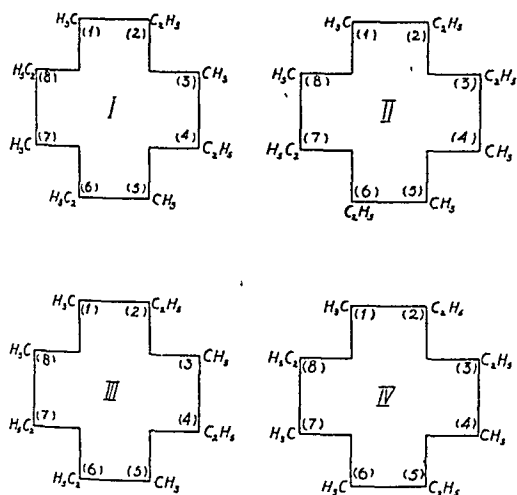
Fig. 2. The four etioporphyrins. Only derivatives of I and III occur in nature. The only difference between I and III is that CH_3 and C_2H_5 in pyrrole ring IV positions (7) and (8) have been interchanged.

Fig. 3. Protoporphyrin IX or 1, 3, 5, 8-tetramethyl-2, 4-divinyl-6, 7-di-propionic acid porphin. 15 isomer types occur. Type IX is derived from etioporphyrin III.

phin or *mesoporphyrin;* of this compound 15 isomers are possible designated I—XV, but only the form IX derived from etioporphyrin III occurs in nature. If the ethyl groups in mesoporphyrin are changed to vinyl one obtains the *protoporphyrins* (tetramethyl-divinyl-dipropionic acid-porphins) of which form IX is found in natural heme. *Hemato-porphyrin* is derived from protoporphyrin by substition of oxyethyl (—CHOH CH_3) for vinyl (—CH = CH_2). By breakdown of heme with strong acids hematoporphyrin is formed while protoporphyrin is formed when weak acids or bacteria are used. A third series of porphyrins which like meso- and proto-porphyrins occur in XV forms are *deuteroporphyrins* (tetramethyl-dihydro-di-propionic acid-porphins) in which two of the H-atoms of porphin remain. Deutero- and mesoporphyrin IX may be formed by bacterial hydration of protoporphyrin IX and occur in the feces.

The porphyrin molecule has a discoid form with the side chains at right angles to its plane. It shows *amphotericity* because of the N in the rings and the COOH in the side chains. It combines with *metals* which

are bound to the N-atoms by main valencies and residual valences under formation of *metallo-porphyrins.* Most important are *iron* compounds (heme from protoporphyrin IX, called protoheme IX; also synthetical hemes are known, *e.g.,* coproheme I from coproporphyrin I) and *magnesium* compounds (in chlorophyll). Heme and chlorophyll are both derived from type III. Also metalloporphyrins with Cu ,Zn, and Co occur in nature (*e.g.,* in urine).

OCCURRENCE IN NATURE

The porphyrins form part of respiratory catalysts through the entire animal kingdom and of the chlorophyll of plants. Heme — protoheme IX — is the prosthetic group of *hemoglobin, myoglobin,* and the *cytochromes;* in lower animals *erythrocruorin* and *chlorocruorin* are found, the latter containing a special heme — *spirographisheme* — differing from *protoheme IX* in containing an aldehyde group instead one of the vinyl groups. Clorocruorin is green in thin layers and red in thick layers for which the blood of animals containing it is green in the small and red in the large vessels (20).

Besides their occurrence bound to proteins in the respiratory catalysts, porphyrins are found in the urine, feces, and bile of higher

animals; in man practically exclusively co-proporphyrin I and III are found here under normal conditions. In the rat protoporphyrin is the main porphyrin of the feces but is only found in traces in the urine. This fecal protoporphyrin is formed by excess synthesis. Also the Harderian gland excretes protoporphyrin (55, 39, 45).

In human erythrocytes free protoporphyrin IX is found. Uroporphyrin-Cu-complexes are found in the red feathers of certain birds (turacus), and uroporphyrins occur in quantity in urine and feces in higher animals under certain pathological conditions. Certain mussels (pteria) store porphyrins in their shells. Copro- and protoporphyrin are formed by yeasts and bacteria.

FORMATION IN THE ORGANISM

Porphyrins are synthesized in the organism from amino acids, and supply of pyrrole rings with the food is not necessary. After feeding of glycine labeled with N^{15}, the N^{15} appears in the pyrrole N of heme (58). Further, if acetate labeled with radioactive carbon is given the radioactive carbon appears in the side chains of heme(4). I and III porphyrins are formed simultaneously, but III in much larger quantities than I, which is due presumably to enzymatic actions (49). The small amounts of I-porphyrins formed and excreted in man under normal conditions are probably by-products without physiological function. The mechanism of the porphyrin synthesis is little known, but presumably we can aquire knowledge by ingestion of porphyrine precursors labeled with tracer atoms to individuals with anomalies of the porphyrin metabolism (cf. 20, a).

The porphyrins may be *endogenous* — i.e., formed in the organism — or *exogenous* — derived from the food. Thus the proto-, meso-, and deuteroporphyrin of human feces are formed from the myoglobin of meat and consequently exogenous, while fecal porphyrins of biliary origin may be endogenous. A porphyrin derived from chlorophyll —

phylloporphyrin — may occur in feces and is absorbed and excreted with the bile. Other *porphyrins do not seem to be absorbed from the intestine* (31, a, 69).

The place of the porphyrin formation, in the adult is the bone marrow; under normal conditions the liver only seems to play a rôle in excretion and destruction, but it may be otherwise in liver diseases.

The daily production of type III porphyrin in the adult human can be calculated from the life time of the erythrocytes which according to recent investigations with a number of different methods (58, 26, 6, 5) is about 120 days. If the blood volume is 5 l and the hemoglobin percentage is 15 the total circulating hemoglobin is 750 g of which 1 : 120 or 6.25 g are broken down daily. As hemoglobin contains 4—5 per cent heme and heme contains 55 parts of iron per 634, the 6.25 g of hemoglobin correspond to 230—285 mg of protoporphyrin — which amount must be synthesized per 24 hours. In comparison the daily excretion of coproporphyrin is negligible — ca. 100 μg with the urine and 300—400 μg with the feces on a diet free from porphyrins.

EXCRETION. THE BILE PIGMENTS

The liver excretes most of the free porphyrins reaching it with the blood — as it does with urobilinoids — and only very small amounts are excreted through the kidneys under normal conditions. By far the larger part of the porphyrins of the organism does, however, not reach the liver in the form of porphyrin as it is found in the erythrocytes as hemoglobin. The protoporphyrin of the blood pigment is not excreted as porphyrin, but most of it is excreted with the bile after conversion to *bile pigments, i.e.,* pyrrol compounds — colored or chromogens — formed in the animal organism by disintegration of heme or allied chromoproteids. The principal bile pigments are *linear tetrapyrroles* or *bilirubinoids,* but also dipyrrylmethenes (bilifuscins and propentdyopents) are found among the natural bile pigments.

Bile pigments are derived from the porphyrins and their chemical nomenclature designed accordingly. If the porphyrin ring is opened at the methene bridge α an α-bilirubinoid results. According to the number of symmetry planes in the molecule of a porphyrin — 2, 1, 0 — the number of bilirubinoids derived varies — 2, 3, 4 respectively. Only in porphyrins without symmetry planes the α, β, γ, and δ-forms are different; if there are symmetry planes two or more of them are identical. Natural bilirubin is derived from protoporphyrin, IX by opening af the α-bridge and is thus proto-bilirubin, IX, α; mesobilirubin is derived in similar way from mesoporphyrin, IX. Also from other porphyrins bilirubins and hemes are derived — e.g., coproheme, I and copro-bilirubin, I, α from coproporphyrin I; these compounds do not occur in nature but have been synthesized. These symmetric synthetic hemes and bilirubins have been used in the effort to understand the reactions of the assymmetric and therefore more complicated natural compounds.

THE BREAKDOWN OF HEMOGLOBIN

According to the classical theory hemoglobin is at first broken down to heme and globin after which iron is given off and the porphyrin ring opened under formation of bilirubin. This theory has, however, been shown to be false in practically every respect. If protoporphyrin is injected into the circulation formation of bilirubin does not take place (70) but if heme or hemoglobin is injected bilirubin is formed, but considerably more rapid and easy after hemoglobin than after hematin (46, 2) which shows that the protein binding is of importance to the breakdown.

The green hemins (18, 65) are important to our understanding of the breakdown of hemoglobin. They can be prepared by oxidation and simultaneous reduction with ascorbic acid (coupled oxidation) (28). By treatment with methanol-HCl they form a biliverdin ester (33). They may be formed by various coupled oxidations (with yeast, liverbrei, or ascorbic acid) and the processes have been

followed with coproheme I (17). Lemberg and co-workers (35, 36, 32) and Engel (10) have followed the disintegration of hemoglobin by coupled oxidation with ascorbic acid under physiological conditions and found that at most 20 per cent of the heme were transformed to biliverdin — the precursor of bilirubin.

Names as pseudohemoglobin (1), verdohemochromogen (34, 35) and choleglobin (35, 36, 32) have been proposed for green hemoglobins and hemochromogens formed under various conditions. To simplify the terminology Kiese & Kaeske (1942) proposed to design the prosthetic group of the green hemoglobins "verde" analogous to "heme" and to use hemo- and verdo- for compounds containing Fe^{++}, hemi- and verdi- for those containing Fe^{+++}; letters after the name of the compound symbolize the process of formation: verdoglobin A (coupled oxidation with ascorbic acid); verdoglobin S (with H_2S = sulphemoglobin); verdoglobin PH (phenylpydrazine); verdoglobin CN (cyanide); if the globin of the compound is denaturated the designation hemochromogen is used. Methemoglobin is hemiglobin. Methods have been worked out for the determination of green hemoglobins (29) and their formation in and elimination from the organism studied (31).

Most authors hold that the porphyrin ring of the green hemins is opened, but this was strongly questioned by Stier (1942) who subjected crystalline copro-I-verdohemin-ester (37) to mild hydration and obtained a porphyrin ester, which showed that the porphyrin ring of the copro-I-verdoheminester must have been intact. Meso- and protohemins behaved in a similar way and were much easier transformed to greens hemins than the symmetric coprohemin. Similar results were arrived at by Kiese (1947, 3) who found that the prosthetic group of verdoglobin NO_2 is the above mentioned spirographisheme containing the porphyrin spirographisporphyrin. Liebecq (38) came, however, to the result that pseudohemoglobin contains an opened porphyrin ring, and that the prosthetic groups of this substance and choleglobin are identical. On the other hand sulphhemoglobin (verdoglobin S) was found to have an unbroken ring. Thus some green hemins may have a closed ring, others an opened one. The difference between the various heme compounds lies in the α-bridge; in heme it is = CH — (methene), in oxyheme = COH —, in oxoheme — CO —, in sulphheme — CS —, and in pseudohemo- and choleglobin it has been broken into = O HO —. Contrary to pseudohemo- and choleglobin, sulphhemoglobin can be re-transformed into hemoglobin by alkaline reduction. Sulphhemoglobin gives no liberation of porphyrin by the action of strong acids, and its iron is not set free by diluted HCl. By the action of concentrated ammonia pseudohemo- and choleglobin are transformed into mono-azo-hemochromogen the α-bridge of which has been replaced by = N —. The action

of ammonia on sulphhemoglobin has not yet been studied. These observations (38) point to an opened ring in choleglobin and a closed one in sulphhemoglobin, but further investigations are needed.

The disintegration of heme depends on the protein to which it is attached. Thus myoglobin gives rise to the formation of the dipyrryl-methene bilifuscin (40) instead of the tetrapyrrole bilirubin. In this connection it is to be mentioned that the so-called propentdyopents — dipyrryl-compounds — are never formed in the organism directly from hemoglobin as claimed by Bingold (3) but only from bilirubin and urobilinoids (14, 8).

It has generally been assumed that the heme of hemoglobin is transformed practically hundred per cent to bilirubin, but as mentioned above at the highest a transformation of twenty per cent was found in *vitro*-experiments under conditions as much alike those in *vivo* as possible (35, 36, 32, 10). In feeding experiments (53) with glycine labeled with N^{15} in a human stercobilin containing N^{15} was found in the feces in two periods, the first starting a few days after the glycine administration and the second about 120 days later corresponding to the life time of erythrocytes (cf. above). The excretion of large amounts of N^{15}-stercobilin a few days after the N^{15} has been absorbed shows that a direct synthesis of bile pigments from precursors of protoporphyrin (glycine) must have taken place; the later excretion of N^{15}-stercobilin corresponds to the disintegration of the hemoglobin formed during the period of N^{15}-administration. Thus the old dogma that all bilirubin is derived from hemoglobin — which has never been actually proved — is wrong; bilirubin has a dual origin, partly from synthesis and partly from hemoglobin disintegration. The synthetic production is estimated to about 15 per cent of the total amount of bilirubin formed under normal conditions, but it may presumably reach 50 per cent under certain pathological conditions (Rittenberg, 1948).

THE PROTOPORPHYRIN OF THE ERYTHROCYTES

The occurrence of protoporphyrin in the erythrocytes was discovered by Hijmans van den Bergh & Hijman (1928) and has later been closely studied (67, 22). In normal persons 15—40 (on average 30) $\mu g\%$ are found in the erythrocytes, in iron deficiency anemia 100—600, and in pernicious anemia normal values. In lead poisoning increased values form a symptom of the same importance as porphyriuria. During treatment of pernicious anemia a characteristic increase in the erythrocyte protoporphyrin takes place almost parallel to the reticulocyte reaction but delayed about a week. The protoporphyrin is for a great deal but not exclusively bound to the reticulocytes. Some erythrocytes show fluorescence (57) (fluorocytes) due to their content of protoporphyrin. The protoporphyrin of the erythrocytes seems to arise directly from synthesis and not to be formed by disintegration of hemoglobin.

PORPHYRINS IN DISEASE. THE PORPHYRIAS

In normal man coproporphyrin is found in the urine in an amount up to 100 μg and in the feces in an amount up to 400 μg per 24 hours with 65—92 % of type I (9, 68 a. 69). Recently uroporphyrin has been demonstrated in minute amounts in normal urine by means of filter paper partition chromatography (52; 42a) but more than traces of uroporphyrin do only occur in the porphyrias. In *liver* diseases the excretion with the urine is increased and that with the bile decreased. Thus coproporphyrinuria is a measure of the liver function as is urobilinuria, but as it is found both in parenchymatous and occlusive jaundice it is of little value in differential diagnosis. The coproporphyrin of the urine may increase to ca. 700 μg and that of the feces decrease to ca. 100 μg per 24 hours in liver diseases. The relation between the I and III types is variable (42, 41, 68) but type III seems to dominate in alcoholic

cirrhosis, type I in most other liver lesions (69).

Intoxications with heavy metals, especially Pb (69), as well as some nitro- and amino-compounds (51) also causes coproporphyrinuria, especially of type III. *In diseases* of the blood (pernicious anemia, hemolytic anemias) as well as in *febrile infections* the coproporphyrin excretion is increased too. In all these conditions both the urinary and the biliary excretions are increased as opposed to hepatic diseases in which the former is increased, the latter decreased (9).

The *porphyrias* are primary anomalies of the porphyrin metabolism. Here the excretion of coproporphyrin is enormous and may reach 100 mg per 24 hours in the urine and 1000 mg in the feces and to this comes an excretion of uroporphyrin of the same magnitude. Most often uroporphyrin dominates, but coproporphyrin may be the main porphyrin (69). Our knowledge of these conditions is principally due to Fischer (1915), Rimington (1936), Waldenström (1937) and Watson (21, 72, 69). Reviews on the subject are found by (63, 62, 66, 9, 60). Here only the biochemical aspects are to be mentioned. In *congenital porphyria* the characteristic porphyrin is uroporphyrin I but also coproporphyrins are excreted in collossal amounts. Here the porphyrins do not occur in the urine and feces only, but also in the blood and tissues, especially in the bones; they are deposited in the skin and makes it sensitive to light. In *acute porphyria,* on the other hand, porphyrins are not deposited in the organs, but only excreted with the urine and feces. Waldenström (1937) described a chromogen and a non-porphyrin pigment, later named *porphobilinogen* and *porphobilin* respectively (64). He was of the opinion that uroporphyrin III and these compounds were characteristic of this disease. Porphobilinogen is intermediary between porphyrins and bile pigments and gives the benzaldehyde reaction like urobilinogens, but it is — like its colored product with the benzaldehyde reagent — impossible to extract from aqueos solutions

with organic solvents. The chemical constitution of porphobilinogen and porphobilin is not known, but they are presumably dipyrroles derived from uroporphyrin (64). It has been assumed that all the uroporphyrin in acute porphyria was of the III type, and that the primary product formed in this disease was not porphyrin but porphobilinogen which by oxidation in the urinary passages or outside the organism was transformed into porphobilin and uroporphyrin III (63, 64). The biochemistry of acute porphyria is, however, more complicated. Thus uroporphyrin I has been found in acute porphyria (60), and the "uroporphyrin III" isolated from patients with acute porphyria has been separated into two porphyrins by chromatography (21, 72) — large amounts of uroporphyrin I and small amounts of a porhpyrin of the III type which was, however, found to contain only seven COOH groups and consequently to be different from uroporphyrin III which contains eight. This "heptaporphyrin" has not yet been studied further; renewed investigations are required to decide the question of the occurrence of uroporphyrin III in acute porphyria, but the main porphyrin in this disease is uroporphyrin I which is also found in quantity in the feces in acute porphyria (72, 47) but was absent from the feces in a case of congenital porphyria (69, 72) studied with adequate analytical technic.

Porphobilinogen has been found in the liver of patients with acute porphyria (47), but porphyrins proper are only found in the excreta in this disease with the exception of small quantities in the liver (21, 47). If the urine in patients with acute porphyria is alkaline only the chromogen is excreted, and the urine becomes colorless (64).

The attacks of acute porphyria may be produced by various influences among which are medicaments (sulphonal, barbiturates) (63), lead intoxication (25), and infections (43). The serum citric acid and the urinary 17-ketosteroids have been found increased in patients suffering from acute porphyria and in their relatives (44).

Idiopatic coproporphyrinuria (69, 72a) is a condition in which large amounts of copro-porphyrin III (up to 6 mg per 24 hours) are excreted with urine and feces from unknown causes; no porphyrin is found in the blood and no uroporphyrin is excreted. The persons affected show no other symptoms.

CHEMICAL PROPERTIES. ANALYSIS

The porphyrins have an *intensively red color* and characteristic absorption spectra which vary somewhat with the solvent and the radicals substituted in the porphine. I- and III-isomers cannot be distinguished by their absorption spectra. Changes in pH gives characteristic variations in the absorption spectra by which the porphyrins may be separated from allied compounds as metallo-porphyrins (7).

The porphyrins show a pronounced *red fluorescence* and characteristic fluorescence spectra. The fluorescence is most often least at the isoelectric point. 0,25 % HCl (pH 1.24) is especially fitted for measurement of copro- and uroporphyrins (50). The fluorescence is nearly identical for the I and III isomers.

All porphyrins are insoluble in water, alcohol, pure ether and acetone. Proto-, meso-, deutero- and coproporphyrins are soluble while uroporphyrins are insoluble in ether containing acetic acid. All natural porhpyrins are easily soluble in chloroform containing alcohol because esterfication takes place and porphyrin esters are easily soluble in chloroform.

The best method for quantitative analysis of porphyrins is generally *measurement of their fluorescence* which is very sensitive and specific. 1 μg gives a distinct fluorescence (50). As standard known solutions of coproporphyrin are used, but also solutions of hematoporphyrin which is obtainable commercially have been proposed (12).

Before measurement the porphyrins are extracted, the extracts purified, and the different porphyrins separated from eachother. *Extraction* with acetic acid-ether makes it possible to separate uroporphyrins (in-

soluble) from the other types, and extraction of the etheral solutions with rising concentrations of HCl allows of separation of the others: *copro-* are extracted with 0.2 % HCl, deutero- with 0.36 %, meso- with 0.60 %, and proto- with 1.9 % (73). I and III isomers can, however, not be separated in this way. Copro-I-esters can be separated from copro-III-esters by 30 % aqueous acetone which at 4° C precipitates I in 1½ hour while III remains in solution (56), but in other cases *chromatography* is necessary to separate I and III. This requires high purification and esterification to give reliable results. The latter is obtained by reflux boiling with HCl-methanol for half an hour. Amounts of coprophyrin of the magnitude 5—10 μg may be separated in I and III by chromatography on Al_2O_3 and eluation with 35 % acetone; copro-III-ester is eluated quantitatively while copro-I-ester remains adsorbed (71). Of other methods for the purification and concentration of crude porphyrin solutions *precipitation* of the porphyrins with Ba- or Ca-salts is to be mentioned (7).

Porphyrins are identified by their methyl ester melting-points and crystal forms (19, 7, 9) but recent investigations have shown that this may deceive (27) because as much as 10—15 % of the other isomer (I or III) is required before the ester melting-point discloses impurity, and — as mentioned above — an ester believed to be uroporphyrin III-ester could be separated into two compounds by chromatography (21, 72). Thus chomatography of purified esters has to be carried out before identification.

Determination in erythrocytes (23). The erythrocytes are separated from the plasma by centrifugation, washed with 0.9 % NaCl several times, and shaked with a mixture of glacial acetic acid (1 part) and ethyl acetate (3 parts) for two minutes. After filtration and washing of the filtrate the porphyrin is extracted with 10 % HCl. The HCl extract is neutralized and extracted with ether. The ether is washed and extracted with 5 % HCl. In this final HCl-extract the porphyrin is

measured either by its spectral absorption or by fluorescence.

Determination in urine (63, 7, 50, 59a): The ether soluble porphyrins are extracted with ether after addition of acetic acid, and the extracts washed several imes after which the porphyrins are extracted from the ether with a small volume of 1 % HCl. In this solution the porphyrins may be identified by their acid absorption spectrum. To secure their identity one may return them to ether by addition of sodium acetate and examine their neutral absorption spectrum in the ether. The aqueous phase contains the uroporphyrins which may be isolated by precipitation with a 12 % solution of $BaCl_2$ or by chromatography on Al_2O_3; the latter may also be carried out directly on the urine after filtration (63). Metalloporphyrins and porphobilin are eluated from the Al_2O_3 with glacial acetic acid which do not eluate the porphyrins themselves. These can be eluated later with concentrated ammonia. Uroporphyrin may also be extracted directly from the urine by ethyl acetate at pH 3.2 (63). After extraction and purification measurement is carried out in a fluorimeter. For preliminary orientation the *urochlor reaction* of Waldenström may be of value: urine containing greater amounts of porphyrins gives after addition of equal volume of concentrated HCl a red color; if a drop of a fresh 3 % solution of H_2O_2 is added the color changes to yellow and grass-green and finally disappears. Recently an improved and more simple and rapid technic of porphyrin analysis has been published in this journal (59, a).

Determination in feces (71): The patient has to be placed on a porphyrin free diet if exclusion of exogenous porphyrin is wanted. The feces are collected and weighed, and their fat is extracted simultaneously with esterification of their porphyrins by treatment with methanol-HCl for two days (Watson & Schwartz, 1941). The esters are then extracted with chloroform which is washed with 7 % ammonia and 10 % HCl; after this filtration, chromatography on Al_2O_3 and measurement is carried out. This method determines also the uroporphyrins which were excluded in older methods based on extraction with ether followed by esterification.

Determination of porphobilinogen (63, 61): The Ehrlich benzaldehyde reaction is used. As this reaction is also given by urobilinogens these are axtracted with ether after addition of acetic acid before measurement. As porphobilinogen does not give the Schlesinger reaction with Zn-acetate a positive Ehrlich reaction with negative Schlesinger reaction points to the presence of porphobilinogen. The colored compound of the benzaldehyde reaction is insoluble in chloroform in the case of porphobilinogen but soluble in the case of the urobilinogens (71). A method for quantitative determination has been proposed (61).

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CHLORIDE ANALYSIS IN BIOLOGICAL FLUIDS.
THE MERCURIMETRIC PROCEDURE OF SCHALES & SCHALES

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Methods for the rapid and exact determination of the chloride content of biological fluids are of great importance in clinical laboratories. The authors would probably give preference to the potentiometric titration or to the iodometric chloride titration of Sendroy (1937) as adapted to protein-containing fluids by Van Slyke & Hiller (1947) for large scale soutine use in busy laboratories. Difficulties in obtaining pure silver iodate according to the specifications laid down by Sendroy have unfortunately so far hampered the general use of the iodometric chloride titration. The standard method in this laboratory has for several years been the open Carius method as applied by Van Slyke and Sendroy (1923).

The mercurimetric titration, using diphenylcarbazone as indicator, introduced in clinical chemistry by Schales & Schales (1941) is less timeconsuming than the Van Slyke-Sendroy procedure. The accuracy is satisfactory and the Schales & Schales method has after extensive trials now replaced the open Carius method in this laboratory.

We have found it convenient to introduce some minor changes in the original procedure sera will be reported. The mercurometric titration has been compared with the Van Slyke-Sendroy method. Recoveries of added chloride have been altogether satisfactory with both methods.

For details regarding the Schales & Schales procedure, readers are referred to the original paper.

We have found it convenient to carry out the titration in 1″ porcelain dishes because the color change indicating the endpoint is more easily observed using these dishes than in 25 ml Erlenmeyer flasks. In sera with intensive yellow color due to yellow pigments like bilirubin the true endpoint might be difficult to observe. For such icteric sera we recommend Folin-Wu tungstic acid filtrates for the titration. The color change at the endpoint is sharper, being a stronger blue in tungstic acid filtrates than in non deproteinized sera and the potassium chloride solution used to standardize the mercuric nitrate solution. It should be noted that the consumption of mercuric nitrate is slightly higher for the tungstate-containing KCl standard solution than when the fluid contains no tungstic acid.

When chloride is determined in Folin-Wu filtrates, the standardization of the mercuric nitrate solution should accordingly be performed on KCl solutions containing tungstate, otherwise the results are likely to run too high.

Table I presents representative data obtained using the above mentioned precautions.

During renal clearance studies of chloride excretion using thiosulfate clearance as a measure of glomerular filtration, it was found that thiosulfate in serum and urine forms a greyish-green deposit and no true endpoint could be observed.

Table I.

	Folin-Wu filtrate: m.ekv Cl/l	Van Slyke & Sendroy	Non deproteinized serum m. ekv. Cl/l	
	Schales & Schales		Schales & Schales	Van Slyke & Sendroy
Serum 1.	108.6		108.4	
	108 6		108.7	
Serum 2.	109.6		109.4	
			109.5	109.5
	109.6		109.6	
Serum 3.	107.5	107.6	107.6	
			107.5	108
	107.4		107.7	
Serum 4.	102.9	102.7	102.9	102.0
	102.9	102.7	102.2	101.9
Serum 5.	103.0		104.5	104.5
	104.8		104.5	
Serum 6.	111.5		111.5	111.5
	111.0		111.5	

Comparative analyses of nondeproteinized sera and Folin-Wu filtrates.
The mercurimetric method of Schales & Schales and the open
Carius method of Van Slyke & Sendroy.

Thiosulfate will also interfere with the Volhard-Harvey (1910) titration in urine due to reduction of silver nitrate. When thiosulfate is present in blood and urine the Van Slyke-Sendroy procedure will give excellent results.

Para-aminohippuric acid, creatinine and inulin do not interfere with the mercurimetric titration.

We have so far limited experience with the mercurimetric titration of urines, but our data confirm the findings of Roberts (1936) that the PH of the fluid should be between 1.5 and 2.0 when the endpoint is reached. This will be attained when the mercuric nitrate (or the mercuric oxide) is dissolved in sufficient nitric acid to give the solution a normality of 0.04 HNO_3. Asper, Schales & Schales (1947) found that it was necessary to have a pH of 4.5 to 6.0 at the beginning of the urine chloride titration and that alkaline urines, especially of low chloride content, would give erroneous results if the right pH was not secured. They used

dilute nitric acid to adjust the pH. Bang (1949) mentioned that Brun recommends a citrate-nitric acid buffer of pH 1.5. In urine from patients taking bromide, the bromide will be determined together with chloride in the Schales & Schales procedure. If the total halogen excretion of such patients is to be determined, attention is called to the fact that for the quantitative determination of bromides with the mercurimetric procedure, the right pH is about 0.8 as found by Tetilek (1938) and McCleary (1942). However, using the pH suitable for chlorides, 1.5 to 2.0, only a slight error is thus introduced in the titration of total halogen in bromide containing urines, probably not exceeding 1—2 % of the total halogen.

CONCLUSIONS AND SUMMARY

1. The mercurimetric chloride titration in biological fluids is rapid, exact and well suited for routine use in clinical laboratories.

2. The use of Folin-Wu filtrates for the titration of icteric sera is recommended. Standardization of the mercuric nitrate solution should then be done against standard chloride solution containing tungstic acid.

3. Thiosulfate as present in blood and urine during clearance studies using thiosulfate as a measure of glomerular filtration, interferes with the mercurimetric titration and no true endpoint can be observed. The open Carius method of Van Slyke & Sendroy is recommended when chloride shall be determined in blood and urine containing thiosulfate.

Para-aminohippuric acid, creatinine and inulin do not interfere with the mercurimetric titration.

4. The optimal pH for the titration of chlorides in urine, pH 1.5—2 will introduce a slight error when total halogen is to be determined in bromide containing urines because the optimal pH for quantitative bromide determination is somewhat lower than for chloride, being about 0.8.

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DIAGNOSIS OF ARTERIOSTENOSIS (ARTERIAL OCCLUSION) WITH THE AID OF PULSE WAVE RECORDINGS

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If we palpate the arterial pulse at a point located beyond a narrowing or occlusion of the arterial lumen, the findings are characteristic. The pulse feels more sluggish; that is to say, it rises and falls more gently and has less "strength" than normally. We recognize this puls again in pulsus tardus et parvus, which indeed constitutes a typical symptom of *aortic valve stenosis*, in which the narrowing is situated at the beginning of the arterial course. Following narrowings located more pheripherally we find a similar pulse. Thus, if *in coarctation of the aorta* we palpate the iliac artery (femoral artery), we find on both sides a similar soft, weak pulse, while the pulse in the radial or carotid artery is of the type which we are accustomed to find in *hypertension*.

If the pulse is recorded graphically, it is possible to make a mathematical-physical analysis of it. The sphymograph affords the classical means of recording the pulse. I have used a piezo-electric sphymograph which has

been described earlier (Porjé 1946). Curves recorded in this manner show pressure variations with time. Since it is now possible without any particular difficulty to record pressures intraarterially with suitable manometers, I have also made use of them. Tybjærg-Hansen worked out the technique and method I have employed. On a curve traced in this way the pressure can be given in absolute values at each point of time.

When the pulse is regular, variations in pressure can be regarded as a periodic function of time, i. e. the time period = the pulse time. Periodic movements of the type encountered in mathematical-physical problems can best be studied with the aid of the Fourier analysis, that is to say, the wave movement is resolved into simple sine vibrations, a fundamental vibration and partials. The period of fundamental vibration is equal to that of time, and the periods of the partials are integral parts of the time period. A curve with many peaks is rich in partials. It is possible to express the part of each

Table I.

Age in years	Number of cases	Mean C_1^2	Standard deviating	
			σ abs	percent of mean
20—29 ...	11	0,666	0,04	5,8
30—39...	13	0,613	0,09	14,6
40—49....	10	0,637	0,03	5,2
50—59....	13	0,670	0,06	9,1
60—69....	8	0,407	0,04	6,3
70— ...	3	0,718		

vibration in the total curve by the square of its amplitude obtained according to the following formula:

$$C_n^2 = \frac{A_n^2}{A_1^2 + A_2^2 + A_3^2 + A_4^2 + \cdots \cdots} \quad \cdots\cdots\cdots \sum_{n=1}^{\infty} C^2 = 1$$

A_n = the amplitude of the nth vibration.

If the curve consists of one single sine vibration, $C^2 = 1$; for the remaining vibrations $C^2 = 0$. If, for example, $C_1^2 = 0.9$, the *fundamental vibration* is dominant, since only, 0.1 remains for the partials. Thus when the heart is functioning regularly, every pulse curve can be analyzed with the aid of the Fourier analysis. Important data can be obtained by this means (see also Porjé 1946) The sine vibration composition of the curve is indicated as mentioned above. If the pulse curve is traced from the femoral artery (iliac artery) of a normal person, C_1^2 is obtained according to Table I. In this connection C^2 for the remaining vibrations is of no particular interest and therefore it has not been included

If the artery is narrowed at any point, the pulse assumes a special character beyond the narrowing, as has been said, palpation alone is often sufficient to demonstrate this If the pulse beyond the narrowing is recorded
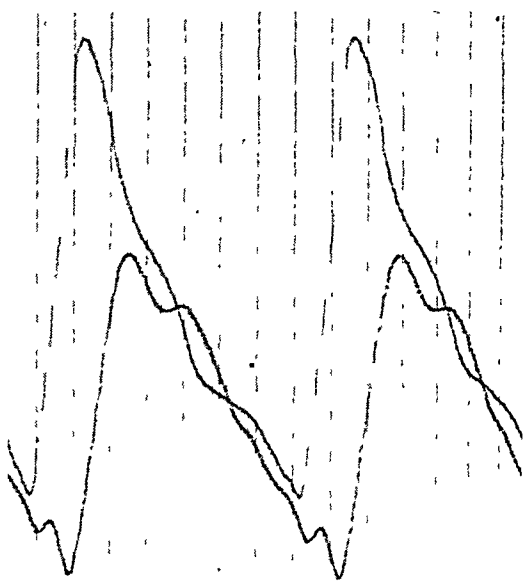
Fig. 1. Case 1. Diagnosis: coarctation of the aorta. 17 year old man. Upper curve; left subclavian artery; lower curve: left iliaca (femoral) artery.
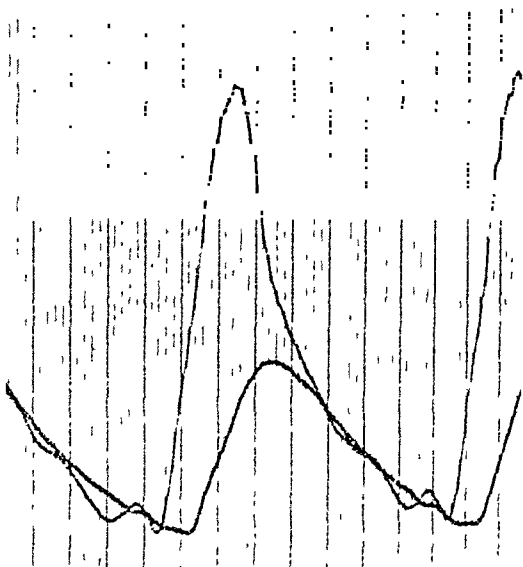
Fig. 2 Case 2. Diagnosis coarctation of the aorta 24 years old man. Upper curve left subclavian artery. lower curve left iliaca (femoral) artery
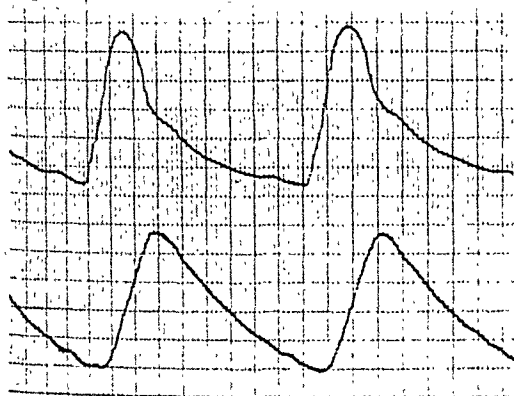
Fig. 3. Case 3. Diagnosis: coarctation of the aorta. 28 year old woman. Upper curve: left subclavian artery; lower curve: left femoral artery.

Fig. 4. Case 4. Diagnosis: coarctation of the aorta. 39 year old woman. Upper curve: left subclavian artery; lower curve: left femoral artery.

according to the methods described above, we obtain a curve which follows certain laws in its deviations from the normal (Porjé 1946). Its contours are softer; the sharp bends and points are absent or much less pronounced. In excessive hypertension and arteriosclerosis similar curves can be found.

Table II.

No.	Age in years	C_1^2
1	17	0,88
2	24	0,92
3	28	0,87
4	39	0,82

If C_1^2 is calculated in accordance with the above, values higher than normal will be obtained. In this investigation detailed studies of the pulse wave have been carried out in cases in which the stenosis was located between the aorta-femoral artery or the femoral artery — art. dorsalis ped.

Coarctation of the aorta. Pulse studies were carried out in 4 cases of coarctation of the aorta. These cases were all clinically certain and the diagnoses were later confirmed by operations. The manifestation of the pulse in the iliac artery (femoral artery) is of special interest in this connection. Thus, we find curves (see Figs. 1—4) recalling a simple sine curve. C_1^2 was calculated for these curves and will be found in Table II. We find values for C_1^2 (see Table II) which are considerably above normal (Table I) and approach 1; in other words, the fundamental vibration is very predominant. The curves resemble a simple sine vibration. In these cases the pulses recorded on both sides have the same manifestation, as was, of course, expected *apriori*, since the stenosis exerted its effect centrally on the pulse wave.

Other cases. In the following I shall report on some cases, in which the narrowing was more peripheral.

Case 5 (see Figs. 5—6). A 60 year old man has been suffering for the past 3 years from cramp in the left calf after walking. Now he can
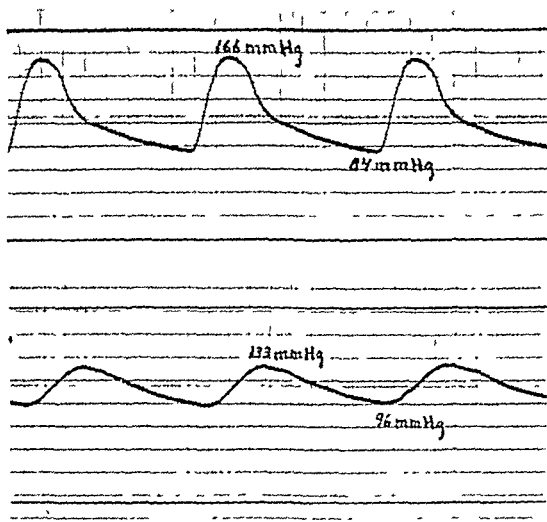
Fig. 5. Case 5. Iliac artery curves. 61 year old man. Diagnosis: stenosis of left iliac artery. Recorded with the manometer of Tybjærg-Hanssen. Upper curve: right iliac artery; ($C_1^2 = 0.50$); lower curve: left iliac artery ($C_1^2 = 0.87$).

only walk 50 meters because of it. In addition to this he feels a stinging sensation and numbness in the leg. At times, when lying on his left side, he also has shooting pains in the left buttock on the outside of the thigh and lower leg, radiating down into the little toe. .

The clinical examinations revealed the following interesting points

Pulsation in the arteries. It was possible to palpate the right iliac artery, the right popliteal artery and the right art. dorsalis ped. The left iliac artery was palpated but felt weaker and softer than the corresponding pulse on the right side. The left poplietal artery and the left art. dorsalis ped. could not be palpated.

Oscillometric index Right femur 1½, left femur 0.5, right lower leg 0 3, left lower leg = 0.

Pulse recordings The right iliac artery curve appeared normal for the patient's age The left iliac artery, on the other hand, assumed the pathological character which appears following a stenosis. The calculation of C_1^2 for the right iliac artery gave 0.50 and for the left 0 87 Thus, in the latter curve the fundamental vibration was deci-

dedly predominant. Oddly enough, the intra-arterial recording revealed the same mean pressure on both sides (112—113 mm Hg), but the amplitude of the blood pressure was decidedly lower on the left (diseased) side (83 mm Hg on the right side as opposed to 36 mm Hg on the left). This circumstance suggests that the vessel was narrowed still further below the stenosis, with a very high resistance to the blood-flow.

Case 6 (see Fig. 7). In another case, described in detail below, different methods gave exactly the same local anatomical diagnosis. The patient is a 48 year old woman who has been suffering from
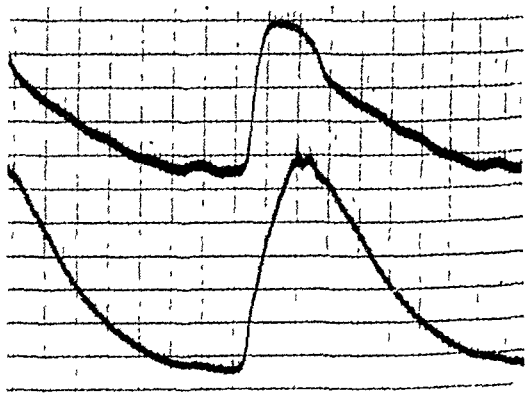
Fig. 6. Case 5. Iliac artery curves recorded with piezoelectric pulse receptor. Upper curve: right iliac artery; lower curve left iliac artery.
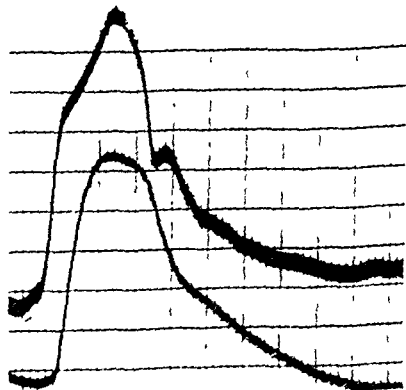
Pulse curves Upper curve right carotic artery lower curve right iliac artery
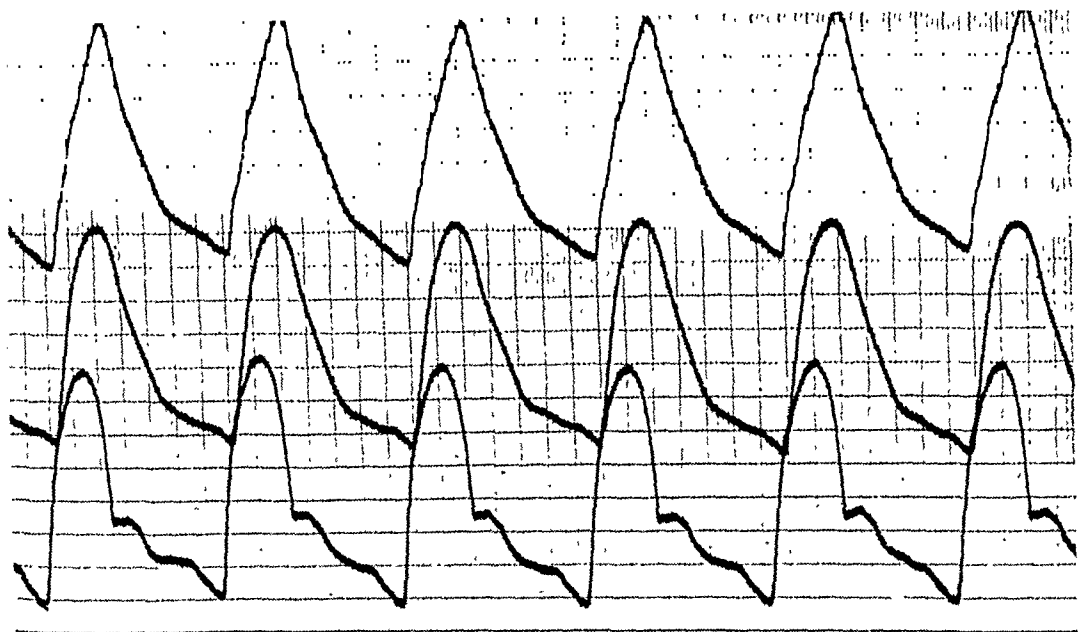
Fig. 7. Case 6. Pulse curves recorded with piezo electric receptors. 48 year old woman. Upper curve: left iliac artery ($C_1^2 = 0.92$. Middle curve: right iliac artery ($C_1^2 = 0.90$). Lower curve: left carotic artery.

mitral heart failure since 1927. This has not, however, prevented her from working for 20 years as a shop assistant. During the six months previous to the examination the patient suffered from a feeling of oppression and a dull pain across the breast and sometimes out towards the upper arms. These symptoms were most noticeable when she was upset and in connection with meals. For many years she had also had a tendency to cold hands and feet, which became numb and cyanotic. In this connection it is of special interest to notice that from the spring of 1947 the patient has suffered from cramp-like dull pains with rigidity and a feeling of numbness in the lumbar region and down the legs, particularly the left one. At first the patient showed symptoms only from the back of the left thigh, but toward the autumn of 1947 she had trouble in the lumbar region also and radiating pains down the left calf, sometimes right down to the lateral malleolus. At times she has also had symptoms from the back of the right leg.

At first she felt the symptoms only under the exertion of climbing hills, but later they were apparent on the level as well. As time went on the symptoms appeared after a walk of only 20 level meters. The symptoms disappeared after a 2—3 minute rest.

The following findings of interest are worthy of mention.

Oscillometric examinations showed marked deflections for both thighs and calves. *Work oscillography*, on the other hand, revealed pathological reactions from the upper and lower legs on both sides.

Skin temperature (according to Dr. Y. Larsson). Heating of the trunk did not involve dilatation of the vessels in the feet.

Aortography at the Serafimer Hospital with percutaneous puncture on a level with the lower part of L_2 showed that there was a short circular constriction of the aorta, a centimeter or two distal to the point of puncture. Below this place

Fig. 8.   Case 7.   Pulse curves recorded with piezoelectric receptors.   60 year old man.   Upper curve:
art. iliaca sin.   Middle curve: art. poplitea sin.   Lower curve: art. dorsalis sin.

Pulse curves recorded with piezoelectric receptors. 60 year old man.   Upper curve: art. poplitea dx.
Lower curve: art. dorsalis ped. dx.
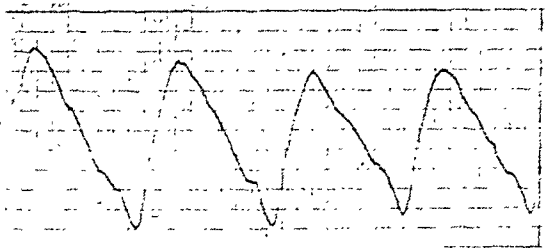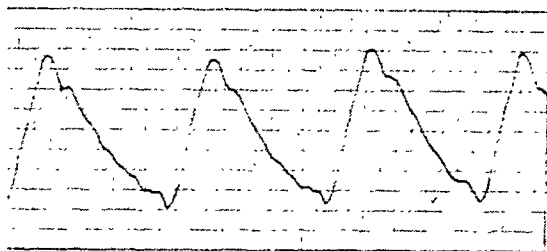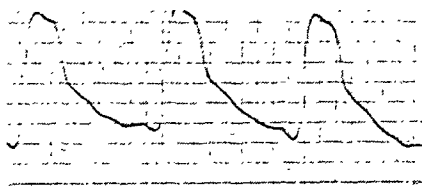
the stenosis was located in the centre of the vessel.

As the patient's blood pressure was normal and she showed no other clinical signs of coarctation of the aorta, the conclusion may be drawn from the pulse curves that the stenosis is either more or less the periphery of the aorta, or that there may possibly be narrowings on both sides of the iliac artery.

Case 7 (see Fig. 8). A 60 year old chauffeur has been suffering from breathlessness for the past 3 or 4 years in connection with any exertion. In addition, since August, 1948, he has shown symptoms of angina pectoris when he walks more than 30 meters. During the last 2 years he has been troubled with pains in the legs, particularly in the left one when walking, the pains have been chiefly confined to the calf and thigh on the left side, radiating up towards the groin. At times the left foot has felt numb and cold. On three occasions the symptoms have been acute and have consisted of an extreme coldness in the left foot and lower

to the division of the aorta the arterial wall was extremely irregular on both sides. On the left side, just above the division of the aorta, there was a rather pronounced shadow in the lumen, about 2 centimeters long. The left common iliac artery had an irregular wall and just before its division into the hypogastric and the external iliac arteries there were short, extremely circular circular stenoses of the vessels. There were no changes on either side of the external iliac or the hypogastric arteries.

Pulse recordings. The *pulse curves* traced bilaterally from the iliac artery showed a "stenosis outline" on both sides.

C^a for the right iliac artery $= 0.90$ and for the left iliac artery $= 0.92$. This finding suggested that

Fig. 9. Case 8. Iliac artery curves. 58 year old man. Upper cuve: right iliac artery $C_1^u = 0.65$. Lower curve: left iliac artery $C_1^u = 0.87$. Diagnosis stenosis of left iliac artery.

g and severe pain in the left groin and about ne decimeter below it.

Examination of the patient revealed that the eft foot was considerably colder than the right.

Oscillometric examinations of the right leg, both high and calf, showed marked deflections, while imilar examinations of the left showed minimal nes

Pulsation in the arteries. The right art. dorsalis ed. was barely palpable, while the left art. dorsalis ed could not definite be palpated.

Pulse recordings Pulse curves were traced from he left iliac artery, the left popliteal artery, the eft art. dorsalis ped., the right popliteal artery, and the right art. dorsalis ped. The pulse curves rom the right side were normal, as was the urve from the left iliac artery. The curves from he left popliteal artery and the left art. dorsalis ed., on the other hand, were typical "stenosis urves".

Thus, on the basis of these pulse curves, a steno-is or occlusion of the left femoral artery might be expected between the groin and the knee. This was subsequently confirmed by the *arteriography*

Case 8 (see Fig. 9) A 58 year old man has had pains in the left leg on walking for the past 3—4 years These began at a point behind the trochanter and radiated downward as far as to the knee, the lower leg and foot becoming completely cold and white, but the patient had no pain in the lower leg The symptoms came when he had ascended only one flight of stairs at an ordinary speed or walked about 200 meters at an ordinary pace. After about 10 minutes' rest the pain disappeared.

Oscillometry gave marked deflections for both the right upper (6.160 mm Hg) and the right lower (5 160 mm Hg) leg, while the deflections for the left were considerably less (2.160 mm Hg or 2.160 mm Hg)

Pulse recordings. The *pulse curves* traced bilaterally from the iliac artery showed that the curve from the right ($C_1^u = 0.65$) was not particularly pathological, while the left had the typical "stenosis appearance" ($C_1^u = 0.87$).

In an attempt at *retrograde arteriography* by means of dissection, a very narrow femoral artery was punctured below the groin The blood pres-
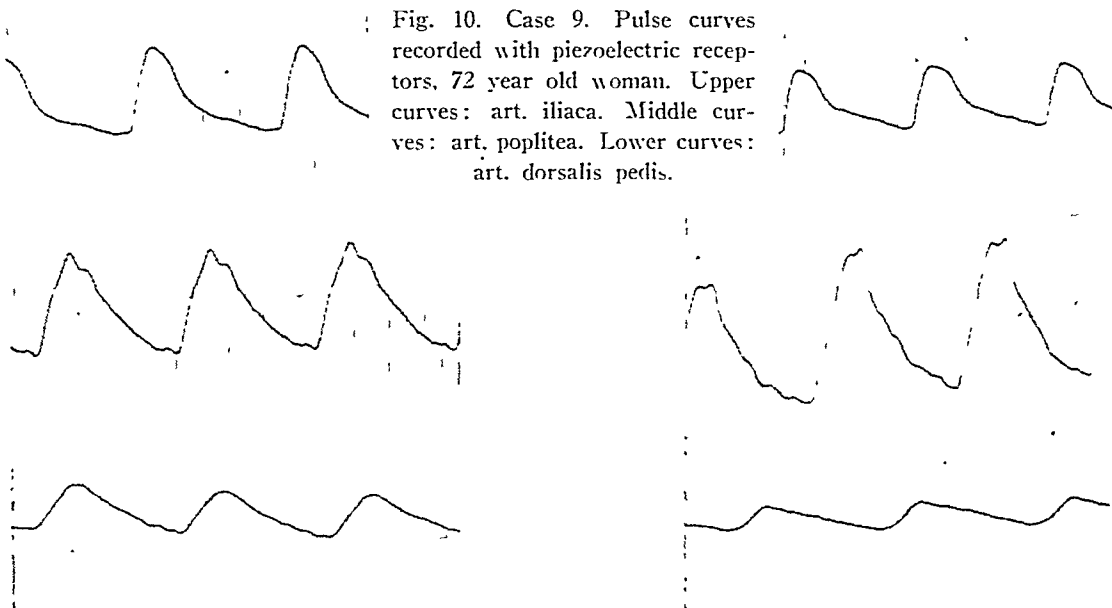
Fig. 10. Case 9. Pulse curves recorded with piezoelectric receptors, 72 year old woman. Upper curves: art. iliaca. Middle curves: art. poplitea. Lower curves: art. dorsalis pedis.

sure was *very low, with small pulsatory pressure variations.* Unfortunately it was impossible to interpret the contrast photography.

Skin temperature. When the patient's trunk was heated, the right foot reacted with practically complete vascular dilatation, while the left became only slightly warmer.

"Stenosis" (possibly occlusion) of the left iliac artery was diagnosed by the pulse curves and confirmed by the other findings.

Case 9 (see Fig 10). A 72 year old woman has had pains in both legs on walking for the past 10 years. She can walk only 50—100 meters and is then obliged to stop because of pains in the legs, particularly the left one. If she tries to walk fast the pains compel her to stop almost immediately.

Examination of the patient revealed the following.

Pulsation in the arteries. The iliac artery pulses were pronounced on both sides, while the art. dorsalis ped. and the popliteal artery felt "soft"

Pulse recordings showed normal iliac artery pulses on both sides, but the *popliteal artery* and the *art. dorsalis ped* had "stenotic pulses" This finding suggests narrowings or possibly occlusions of the arteries on both sides between the femoral artery and the art dorsalis ped

Arteriography of the left femoral artery with contrast confirmed the diagnosis. Arteriography of the right femoral artery was not undertaken

Case 10. One year before the examination a 66 year old man began to feel tired in the right calf During December 1948 his symptoms increased He then began to have further difficulty in the form of pain and shooting pains in the calf so that he sometimes had to sit down and rest. When he reported to us he could walk only 300—400 meters without any cramp

Examinations revealed the following

Heart· physical examination including ECG: normal. *Blood Pressure.* 190/100...   .. ..

Pulsation in the arteries Palpation showed that the femoral artery was normal on both sides The left popliteal artery was distinctly palpable Whether or not the right popliteal artery could be palpated was uncertain.

Pulse recordings The iliac artery curves were normal on both sides, as were the curves from the left popliteal artery. The right popliteal artery curves showed *pronounced stenotic characteristics* This finding suggests *occlusion or stenosis of the femoral artery above the knee*

Arteriography of the right femoral artery revealed an occlusion of the lower part of the femoral artery for about 5 centimeters.

Case 11. A 40 year old engineer who was formerly well has had increasing difficulty for the last 3 years in both legs in the form of cramps and pains in the calves after walking. He can now walk only about 200 meters on level ground and only about 50 meters in hilly country before the onset of the aforementioned symptoms. In addition his feet get white and icy cold. If he rests for about 15 minutes the symptoms disappear. They become worse in cold weather. He smokes a good deal and has been a smoker since the age of 18. Now he is smoking about 20 cigarettes a day.

Examination showed normal *cardiac findings* (including ECG and cardiac x-ray).

Oscillometric examinations gave higher deflections for the arms than for the lower extremities (the upper extremity 7—8/120 mm Hg and the lower 1—2/120 mm Hg).

Measurement of the skin (according to Dr. Y. Larsson) after heating the trunk gave an increase in the temperature of the toes though not as much as in normal persons.

Pulsation in the arteries. The iliac artery was palpated normally on both sides. The popliteal artery and dorsalis pedis felt weak.

Pulse curves recorded bilaterally from the iliac artery were of normal appearance, while bilateral tracings of curves from the popliteal artery and the dorsalis pedis were of "stenotic type". (C_1^2 art. iliaca dx. $= 0.66$; C_1^2 art. poplitea dx. $= 0.84$; C_1^2 art. dorsalis ped. dx. $= 0.97$). These findings suggest bilateral occlusion or narrowing of the femoral artery.

Arteriography of the right artery showed total occlusion at the transition to the popliteal artery. A collateral circulation had developed and was functioning fairly well.

DISCUSSION

Physical and Physiological aspects. Rigid stenosis of an elastic tube produces certain characteristic changes in a pressure wave passing through the tube. This is easy to demonstrate mathematically. Thus, a stenosis of this description gives rise to:

1) standing waves in front of the stenosis;

2) a considerable decrease in the amplitude and a phase lag of the wave following the stenosis;

3) *characteristic changes in the wave profile following the stenosis, due to the fact that there is a greater decrease in the amplitudes of the "higher frequencies" than there is in those of the lower.*

If there is complete occlusion of the vessel and collaterals have developed, changes in the pressure waves are obtained beyond the occlusion in the same way as beyond a stenosis. In physical respects the relatively narrow and inelastic collaterals are equivalent to a rigid stenosis.

Clinical and Diagnostic Aspects. When there is clinical reason to suspect a narrowing or occlusion of the artery lumen, the characteristic pulse curves, which in such cases are recorded beyond the stenosis or occlusion, give the diagnosis. Probably it will not be possible to decide whether it is a question of a stenosis or a total occlusion with collaterals. *When stenoses are located in the aorta or iliac artery, it can be particularly difficult to obtain a diagnosis with the usual clinical examination methods. In such cases a diagnosis can be made very easily by recording the pulse.*

Further it is of interest that in cases in which the pulse has not definitely been palpable, it has been possible to record it with considerable deflections by adopting the particularly sensitive piezo-electric technique. This means that the method can be used to advantage even in the diagnosis of stenosis or occlusion of the peripheral arteries.

SUMMARY

The author describes a number of cases with narrowings or occlusions of the arterial lumen. The characteristic changes of the pulse wave which arise in such cases are discussed from the physio-physiological point of view. The author describes a method by which it is possible to diagnose and lokaliz narrowings or occlusions of the arteria lumen.

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POLAROGRAPHIC DETERMINATION OF MAGNESIUM IN SERUM BY AID OF 8-HYDROXYQUINOLINE

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In recent years there has been an ineasing interest in the study of magnesium blood, urine and tissue under pathological onditions, since the metabolism of magneum seems to be disturbed in several disetes. In clinical laboratories there has thus risen a greater need for easier and more ipid magnesium determination methods. he usual methods for magnesium determintion include precipitation with compounds ich as 8-Hydroxyquinoline, isolation and urification of the precipitate, and finally olorimetric or titrimetric determination. he methods are time-consuming, involve teps with danger of loss, and are not very ccurate. It has seemed desirable, therefore o seek new methods for magnesium determination. The polarographic methods have nany advantages in analytical chemistry; hey are in general rapidly performed and . high degree of accuracy may be attained vith small amounts of material.

The direct polarographic determination of magnesium is not possible because magneium gives a very poorly defined wave in olutions of $(CH_3)_4NJ$ as the supporting electrolyte (Kolthoff & Lingane 1946). Quite mother principle has to be applied, i. e. the polarographic determination of organic compounds can be used indirectly to determine inorganic ions. The procedure consists in adding a known amount of an organic compound which can be determined polarographically, and which can also be precipitated by the ions in question. After the precipitation, the amount of organic reagent left in the solution is determined.

This principle has been applied for determination of magnesium by means of 8-Hydroxyquinoline (Stone & Furman 1944) and for calcium by means of picrolonic acid (Cohn & Kolthoff 1943). Carruther (1943) first found that 8-Hydroxyquinoline was reduced at the dropping mercury cathode. Two diffusion current waves were found in the polarogram (Fig. 1). The first wave had an $E_{\frac{1}{2}} = -1.39$ volts against saturated calomel electrode. The wave was found to be constant with concentrations in the range under consideration. The wave did not shift with slight changes in pH, and the diffusion current was found proportional to the concentration. This wave could therefore be used in quantitative determinations. The second wave in the polarogram had an $E_{\frac{1}{2}} = -1.61$ volts vs. s.c.e. The half potential wave shifted slightly with the concentration and considerably with slight changes in pH. This wave therefore could not be used in analytical methods.

REAGENTS AND APPARATUS

8-Hydroxyquinoline was obtained from Eastman Kodak Company, N. Y. It was recrystallized four times from alcohol — water mixtures. The standard solution used was 5.10^{-3}M 8-Hydroxyquinoline in 5 per cent alcohol. This solution was made by dissolving 383 mg 8-Hydroxyquinoline in 25 ml 96 per cent alcohol and subsequent dilution with distilled water to 500 ml volume. On standing at room temperature it is slowly decomposed. New solution was made every two weeks.

Ammonia-ammoinum chloride buffer was made by mixinig 400 ml 0.1 N NH₄Cl p.a. (from Baker, N. Y.) with 55 ml conc. NH₃ p.a. (from Merck, Darmstadt) followed by dilution to 500 ml with distilled water. The buffer was then adjusted to pH = 10.6 with a pH-metcr, "Radiometer" and a glass electrode. (The buffer is 1.55 M in total ammonia and 0.08 M in ammonium chloride).

A standard 0.01 N MgCl₂.6H₂O solution was made from MgCl₂.6H₂O p.a. Merck, Darmstadt. The chloride content was checked by Volhard titration and by electrometric titration (Lehmann 1944). All reagents were tested for purity in the polarograph. A polarograph from A/S "Radiometer", Copenhagen was used for all determinations. The capillary had the following characteristics in ammonia-ammonium chloride buffer (pH = 10) at — 1.0 volt against saturated calomel electrode: Mass: 1.66 mg per second Drop time: 3.80 sec. H = 67.7 cm mercury pressure. The polarographic cell was immersed in a waterbath at 25° C. Oxygen was removed from the cell by the passage of purified nitrogen through the solution for 15 minutes, and polarography then took place in a closed cell. Most of the polarograms were taken at ⅕ sensivity

PROCEDURE

The method, involves the following steps:

1. Precipitation of calcium from serum by oxalate (Sendroy 1944).
2. Supernatent ashed, and the ash dissolved in HCl.
3. Precipitation of ash solution with 8-Hydroxyquinoline in ammonia—ammonium chloride buffer at pH = 10.

4. Polarographic determination of 8-Hydroxyquinoline left in the solution, without removing the precipitate. A blank on the 8-Hydroxyquinoline solution is always run simultaneously in the polarograph.

To 2 ml serum there were added 4 ml distilled water and 1 ml saturated ammonium oxalate. After mixing and standing overnight the precipitate was removed by centrifugation, the supernatant
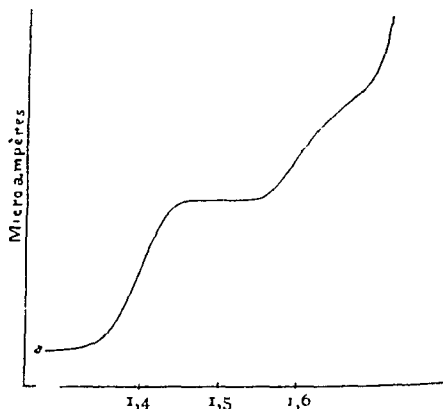
Fig. 1. Polarogram of 8-Hydroxyquinoline showing the two waves. C = 1.10^{-3} mole per liter at pH = 10.
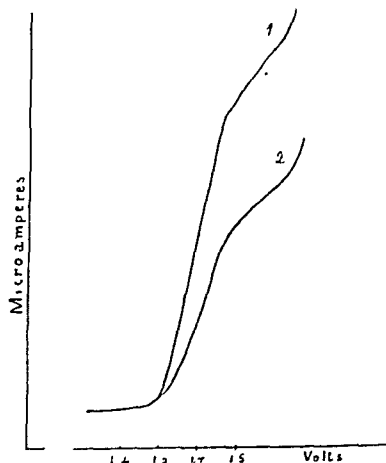
Fig. 2. Effect of magnesium on the polarogram of 8-Hydroxyquinoline. C = 1.10^{-3} mole per liter. 1. No magnesium added. 2. Addition of 2.4 microgram magnesium per ml.

was transferred to a 15 ml silicon dish and 5 drops 50 per cent H₂SO₄ added. Evaporation to dryness on a steam bath, then in a muffle furnace at 110° C for 20 minutes and gradually heating to 500—600°C. At this temperature the sample was ashed for 2—4 hours or more. After cooling, the ash was dissolved in 2 ml 0.5 N HCl and transferred to a 10 ml volumetric flask. 4 ml buffer pH = 10.6 was added and then 2 ml 8-Hydroxyquinoline standard solution added slowly while stirring vigorously. The solution was diluted to the mark with distilled water, then allowed to stand for 2 hours or more and frequently shaken. Polarograms were taken at ⅕ sensivity. A blank containing 2 ml of the 8-Hydroxyquinoline solution and the reagents was run simultaneously.

Calculation: The reduction in wave height of the polarogram, produced by the precipitation of Mg oxine is measured (Fig. 2). The Mg content is then read on a standard curve.

DISCUSSION

The method has been applied to series of analyses of serum and muscle tissue for magnesium, and has worked very satisfactorly. During these analyses it has become apparent that the polarographic method for Mg offers several advantages over titrimetric and colorimetric methods. But the described method also gives a typical example of the difficulties involved in application of polarographic methods to biochemical analyses. It must be admitted that some of the elegance of the technique is lost, because it is necessary to subject the material to several operations before polarographic determination can be made. As calcium interferes in amounts over 20 microgram pr. ml (Stone & Furman 1944) it must be removed in serum analyses. But this step does not complicate the method as the precipitate can be used for calcium determination and the supernatant for mag-

nesium. In tissue samples of 0.2—0.4 g removal of calcium is not necessary. The other electrolytes; sodium, potassium, phosphates and sulphates do not interfere in the range of concentration found in biological material. The ashing procedure is necessary to remove polarographic reducible organic matter from the sample and to remove the oxalate reagent. The polarographic determination however can be carried out without removing the magnesium oxine precipitate.

For polarographic determination of organic compounds such as 8-Hydroxyquinoline, the analytical conditions must be carefully standardized (Kolthoff & Lingane 1946). The precipitation of magnesium oxine also demands strictly standardized conditions (Miller 1940, Yoe & Sarver 1941). The maximum solubility of the magnesium oxine precipitate is 0.4 microgram pr. 10 ml solution (Stone & Furman 1944), equal to a maximum error of 0.2 per cent in the analyses.

Standard solutions of magnesium have given a straight line curve relationship for

Table I.

Amounts of magnesium added	Found in analysis	Balance
24 microgram	24.6 microgram	+ 2.5 %
24 —	24.1 —	+ 0.4 %
24 —	23.8 —	— 0.8 %
24 —	23.4 —	— 2.4 %
24 —	24.0 —	0 %
24 —	24.4 —	+ 1.7 %
24 —	23.8 —	— 0.8 %
24 —	25.0 —	+ 4.0 %
	Mean: 24.1 microgram	+ 0.5 %

Recovery of known amounts of magnesium added to 1 ml serum.

10 to 50 microgram of magnesium. The accuracy has been found $\pm$ 2.5 per cent. The recovery of known amounts of magnesium added to serum analyses is shown in Table 1.

Determination of magnesium in serum on 11 students shows a mean value of 1.92 m. eq./l. with a standard deviation of 0.05 m. eq./l. The highest value found was 2.23 m. eq./l. and the lowest 1.72 m. eq./l.

SUMMARY

A polarographic method for determination of magnesium in biological material by the polarographic reduction of 8-Hydroxyquino-line, without removing the Mg oxine precipitate is worked out. The amounts needed are 2 ml serum or 0.2—0.4 g tissue. The method has worked very satisfactorily in series of analyses.

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ENDOGENOUS "CREATININE" CLEARANCE

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Modern photoelectric colorimeters have made possible exact determinations of the low concentrations of creatinine in plasma. However, not only creatinine but also some other chromogenous substances in blood will give the picrate reaction of Jaffé. These other substances are not excreted in the urine. Miller and Dubos (1937), with an enzymatic method, showed that in normal plasma creatinine makes up from 81 to 100 per cent of the whole chromogenous material, and in nephritic plasma from 69 to 100 per cent. In the red cells creatinine makes up only 40 per cent of the chromogenous material. But in urine they found that almost all of the chromogenous material (98 per cent) is creatinine. As a consequence the endogenous "creatinine" clearance determined with Jaffé's reaction must be found a little lower than it really ought to be.

In Table I are listed the results obtained by previous authors regarding endogenous creatinine clearance.

Addis (1948) highly recommends endogenous "creatinine" clearance for clinical work. His average normal value is 101 ml per minute with a variation coefficient of only 12.6 per cent (Heat precipitation — Jaffé's reaction). After excision of varying amounts of kidney tissue in rats he found a very good correlation between remaining amounts of kidney tissue and endogenous "creatinine" clearance. When urea clearance was used instead of endogenous "creatinine" clearance, the correlation was less satisfactory.

For practical purposes Addis has recommended a determination of creatinine in plasma only, as the urinary excretion in each individual is nearly constant.

From Table 1 it is seen that there is, in normals, a very close relation between endogenous "creatinine" clearance and the glomerular filtrate. In renal diseases plasma contains relatively more of the non-creatinine chromogenous material. This will tend to lower the endogenous "creatinine" clearance. But on the other hand high plasma concentrations as a result of decreased renal function will tend to increase the clearance, as one must expect some tubular excretion of creatinine. These two processes may counteract each other to some degree. It appears however from the table that in glomerular nephritis endogenous "creatinine" clearance is found to be a little higher than the glomerular filtration rate.

Comparing urea clearance and endogenous "creatinine" clearance, the latter has the advantage of being independent of diuresis and to a higher degree also of dietary protein and other extrarenal factors. The

Table I.

Author	Methods	Results
Popper and Mandel 1937	Jaffé reaction Picric acid filtrate[1]	End. "creat." cl. 80 – 180 ml per minute and 30 % lower than exog creat.cl. a. m. Rehberg
Miller and Winkler 1938	Enzymatic Tungstic acid filtrate	7 normals: End. creat. cl./in. cl. = 1.0 (0.8 – 1.8) 4 nephritics: End. creat. cl /in.cl = 1.38 (0.9 – 1.7)
Steinitz and Türkand 1940	Jaffé reaction Picric acid filtrate	11 normals: End. "creat." cl./in.cl. = 1.03 (0.73 – 1.17) 6 nephritics: End. "creat." cl./in.cl = 1.37 (1.04 – 1.73)
Smith, Finkelstein and Smith 1940	Jaffé reaction Picric acid filtrate	5 normals + 2 hypertensives: End. "creat." cl./in.cl. = 1.19 (1.01 – 1.42)
Smith, Finkelstein and Smith 1940	$BaCO_3 - Fe_2(SO_4)_3 -$ filtrate	5 normals + 2 hypertensives: End. "creat." cl./in.cl. = 0.94 (0.82 – 1.08)
Brod and Sirota 1948	Jaffé reaction Tungstic acid filtrate	14 normals: End. "creat." cl./in.cl. = 1.0 (0.88 – 1.10) 7 normals: End. "creat." cl./thio-sulf.cl. = 0.95(0.84 – 1.01) 13 nephritics: End. "creat."cl./in cl. = 1 04(0.89 – 1.25) 9 nephritics: End. "creat."cl./thio-sulf.cl. = 1.10(0.77 – 1.63)

[1] It seems that with picric acid some of the chromogenous material is precipitated.

plasma concentration of creatinine is very constant throughout the day. This makes longer clearance periods possible, and only one venous puncture may suffice.

The normal creatinine concentration in plasma lies around 1 mg per 100 ml. Variations have been found between 0.6 and 1.7 mg per 100 ml, usually not above 1.20 (Allinson 1945, Brod and Sirota 1948, Popper and Mandel 1937, Steinitz and Türkand 1940, Tierney and Peters 1943, Addis 1948).

The chemical determination of creatinine is considerably less complicated than the determination of urea.

The *object of this paper* is to test the clinical value of endogenous "creatinine" clearance in comparison with urea clearance. It further aims to relate the endogenous "creatinine" clearance to the glomerular filtration rate determined by inulin and sodium thiosulfate.

MATERIAL

Some of this is drawn from investigations of the renal blood flow in normals and cardiac patients, partly under induced hypoxaemia and muscular activity (Blegen and Aas 1949), partly also after intravenous

administration of tetraethylammonium bromide (Blegen and Aas 1949). In this manner variations in clearance values have been induced, both in the individual and from one individual to another.

The standardized technique for clearance determinations given by Homer Smith and collaborators (1938 and 1945) has been used. Constant infusion of inulin, sodium thiosulfate and p-sodium-hippurate has afforded an approximately constant plasma concentration. Exact urine volumes have been secured through an indwelling catheter and manual expression over the symphysis after injection of water and air. The diuresis has been sufficiently raised by water drinking. Most clearance periods have lasted 10 minutes, and up to 6 periods have been used in each case.

In a little group of 15 normal individuals, all young men and women, the bladder was emptied spontaneously. Here a single infusion of inulin was given. The clearance periods in these instances lasted half an hour.

All these clearance values have been corrected to a standard surface area of 1.73 m².

The material further comprises 36 cases with renal disease, mostly acute and chronic glomerular nephritis. In these instances only endogenous "creatinine" clearance and urea clearance have been determined. All patients have been fasting. The clearance periods here lasted 2 hours. The men have emptied their bladders spontaneously, and only the women have been catheterized, without instillation of water and air. One blood specimen has been taken in the middle of the clearance period. These clearance values have not been corrected for surface area.

METHODS

Inulin has been determined by the diphenylamine method as described by Corcoran and Page (1939) and Alving, Rubin and Miller (1939).

Thiosulfate has been determined by a modification of the method given by Newman, Gilman and Philips (1946). This modification has been

described by C. Brun (1947). For more detailed description the reader is referred to an earlier paper (Blegen, Örning and Aas 1949).

Urea has been determined in a Somogyi filtrate by the manometric method of Van Slyke.

Endogenous "creatinine" has been determined with the picrate reaction of Jaffé after Folin and Wu (1919). Hemolysis must be avoided. Protein precipitation has usually been done in dilution 1 : 5 through sodium tungstate and sulfuric acid. Small dilutions like this are an advantage. At the start of the investigations a Somogyi precipitation with a plasma dilution 1 : 10 was used. This seemed to give a little higher value for creatinine.

The urine has usually been diluted 1 : 50.

All colorimetric determinations have been made in a Klett Summerson photoelectric apparatus, green filter. For creatinine the colorimetration has been performed after 15 minutes. The color is stable for about 30 minutes (Bonsnes and Taussky 1945).

RESULTS

The normal "creatinine" clearance determined in 37 individuals and 67 clearance periods averaged 105 ml per minute ± 21 with a range of 67 to 163 ml per minute. Average normal urea clearance in 37 indi-
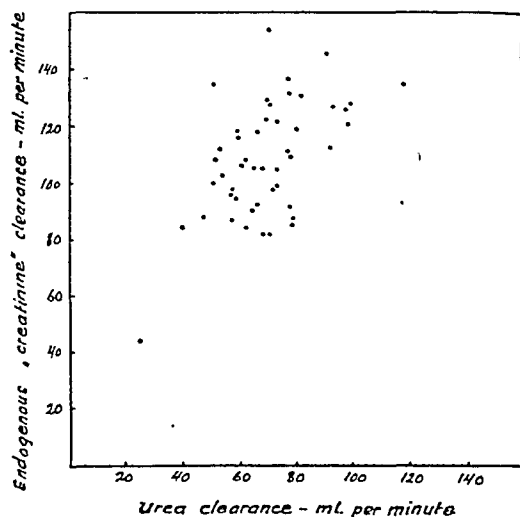
Fig. 1.
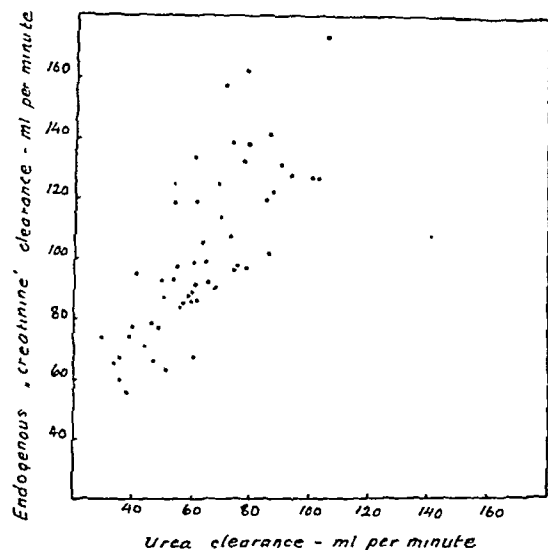
Fig. 2.
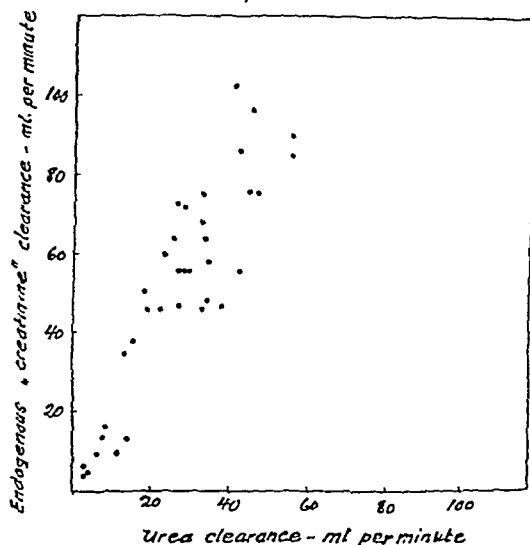
Fig. 3.

viduals and 41 clearance periods was 74 ml per minute $\pm$ 20 with a range of 40 to 118 ml per minute.

In 40 normals and 51 clearance periods the average ratio endogenous "creatinine" clearance/urea clearance was 1.55 with a standard deviation of $\pm$ 0.37. From Fig. 1 it will be seen that there is a fairly good correlation between the two values. The coefficient of correlation is 0.52 and the coefficient of regression is 0.47. The range the values for "creatinine" clearance is than for urea clearance.

In 30 cardiac patients (valvular di and 54 clearance periods the same ratio 1.63 with a standard deviation of $\pm$ 0 A good correlation will be seen in Fig The coefficient of correlation is 0.82, coefficient or regression 0.50.

In all clearance periods above, the di exceeded 2 ml per minute.

In 36 patients with renal disease, mostly acute and chronic glomerular nephritis, the ratio was 1.92 with a standard deviation of $\pm$ 0.48. In 23 periods the diuresis was less thar   minute, and in 13 more than

Fig. 4.

Fig. 5.
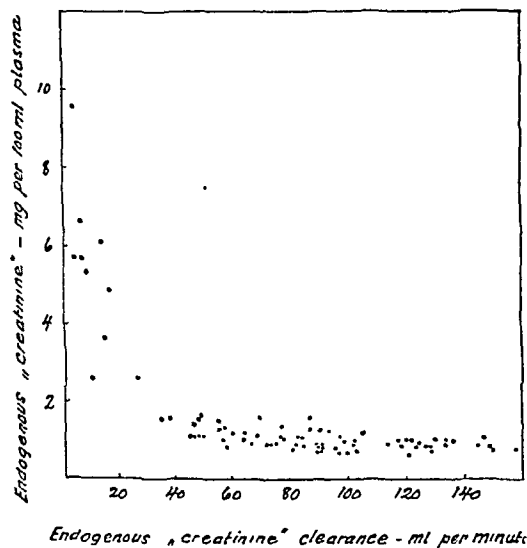
Inulin clearance, and later sodium thiosulfate clearance, have been used as a measure of the glomerular filtration rate. In 37 normals (men and women) and 67 clearance periods the average inulin clearance was 126 ml per minute ± 25, with a range of 74 to 184 ml per minute (Goldring and Chasis 1944 found 131 ± 22 for men and 117 ± 16) for women).

In these, 37 normals simultaneous determinations of endogenous "creatinine" clearance and inulin clearance were made in 103 periods. The average ratio was 0.84 with a standard deviation of ± 0.14. Fig. 5 shows a good correlation. The coefficient of correlation is 0.73, the coefficient of regression 0.90. The range of the normal "creatinine" clearance values is less than for inulin clearance. The deviation of the average ratio from 1.00 is eleven times the standard error, and therefore significant.
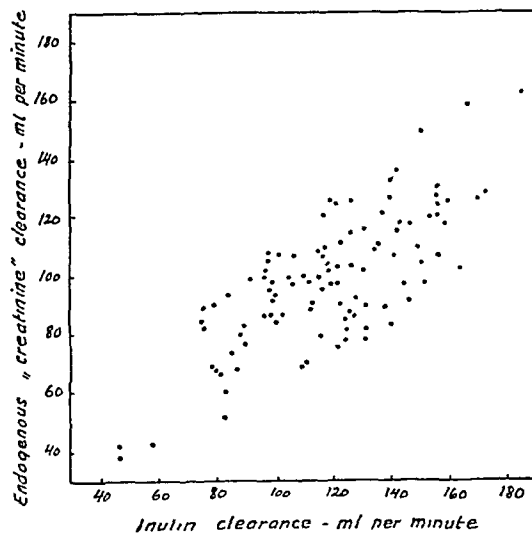
In the normals the ratio inulin clearance/ urea clearance was 1.70 (Smith, Goldring and Chasis 1938 found 1.76). As mentioned above, the normal ratio endogenous "creatinine" clearance/urea clearance was 1.55, i. e. significantly lower.

In 27 cardiac patients, partly under induced hypoxemia or muscular activity, the average ratio endogenous "creatinine" clearance/inulin clearance in 100 periods was 0.89 with a standard deviation of ± 0.16. A good correlation is seen in Fig. 6. The coefficient of correlation is 0.71, the coefficient of regression 0.95. Also here the range of the "creatinine" clearance values is less than for inulin clearance. The deviation of the average ratio from 1.00 is seven times the standard error, and therefore significant.

In 13 cardiac patients and two normals (85 periods), the average ratio endogenous "creatinine" clearance/thiosulfate clearance
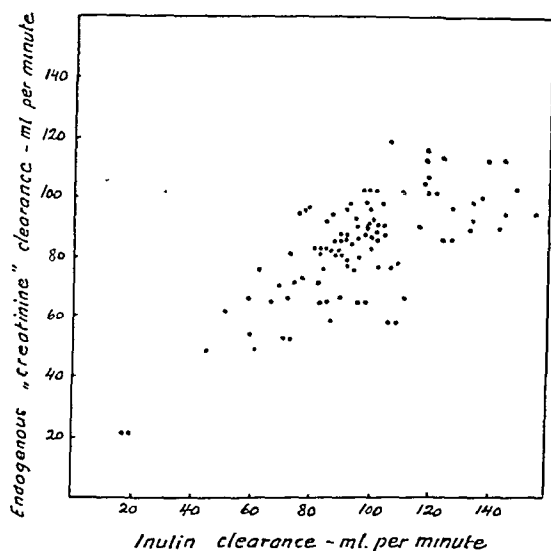
Fig. 6.
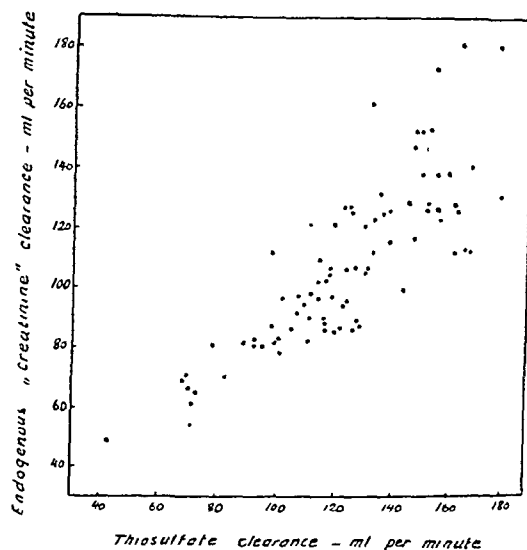
Fig. 7.

was 0.89 with a standard deviation of ± 0.13. Fig. 7 shows a good correlation. The coefficient of correlation is 0.80, the coefficient of regression 0.79. The deviation of the average ratio from 1.00 is eight times the standard error, and therefore significant.

It is apparent that in these patients with valvular disease, the ratios endogenous "creatinine" clearance/inulin clearance and the endogenous "creatinine" clearance/thiosulfate clearance are equal, i. e. 0.89.

COMMENTS

The results show that in normals and in cardiac patients, endogenous "creatinine" clearance lies about 60 per cent above urea clearance. Direct comparison has here been possible when only urea clearance values with diuresis above 2 ml per minute have been considered. There is a good correlation between the two values. Some few values fall outside, examples of the well

known but largely unexplained fluctuations both in urea and to a lesser degree in creatinine clearance.

As a result of the experimental conditions with the effect of induced hypoxemia, muscular activity and administration of tetraaethylammonium bromide, normals and cardiac patients have come down to a urea clearance of 30 ml per minute. Throughout all this range, from 30 ml per minute and up there is a distinct paralellism between endogenous "creatinine" clearance and urea clearance. The range of the values is less for "creatinine" clearance than for urea clearance.

In renal diseases special problems arise. Firstly, urea clearance values with diureses less than 2 ml per minute have not been discarded. The clearance values have been computed as maximum clearances. As urea clearance falls with declining diuresis (when this is lower than about 2 ml per minute)

and "creatinine" clearance is independent of diuresis, the values are not directly comparable. Secondly, in renal diseases there will frequently be more non creatinine chromogenous material in plasma than under normal conditions. This will tend to lower the endogenous "creatinine" clearance. Thirdly, high plasma concentrations of creatinine may lead to tubular excretion with resulting increase in clearance. Creatinine clearance with high plasma concentration, after oral administration of creatinine, lies about 30 per cent above the simultaneous glomerular filtration rate (Shannon 1935).

It is hard to tell exactly how all these factors will influence the ratio endogenous "creatinine" clearance/urea clearance. In this material the average endogenous "creatinine" clearance in renal disease is almost twice the urea clearance. It follows that endogenous "creatinine" clearance lies relatively higher in renal disease than in normals and cardiac patients. But also here there is a good correlation between the two values. Some spreading of the values exists, but this is practically unimportant, because of the low absolute values. Of real importance is the fact that in each single case where urea clearance is significantly reduced, the endogenous "creatinine" clearance is equally reduced.

As for the ratio endogenous "creatinine" clearance/urea clearance, a statistical analysis shows that the difference in the coefficient of regression between normals, cardiac patients and renal patients is not significant. For all three groups as a whole the coefficient of correlation is 0.82 and the coefficient of regression is 0.58. From a known "creatinine" clearance the corresponding urea clearance can be estimated according to the equation: Urea clearance $= 4 + 0.58 \times$ creatinine clearance, i.e. for practical use: urea clearance $= 0.6 \times$ creatinine clearance.

In rats direct correlation between endogenous "creatinine" clearance and the weight of functioning kidney tissue has been demonstrated by Addis (1948). As Addis also has pointed out, the clinician usually has no nead for a subtle measurement of functional impairment. It is enough for him to know if almost all kidney tissue is destroyed, a large part, a moderate part or almost none.

Our results agree with those of Addis also in that the plasma concentration of endogenous "creatinine" gives quite a good idea of the extent of the kidney lesion. Concentrations above 1.3 mg per 100 ml seem to indicate a reduced function.

It will be apparent that in these investigations the endogenous "creatinine" clearance does not give much more information than urea clearance. And much can be said to the benefit of the latter. One must admit that the excretion of urea constitutes one of the most important quantitative functions of the kidney. But aside from the theoretical advantage of creatinine clearance, that it closely corresponds to an important part of the kidney function, i. e. the glomerular filtration rate, there are other factors: Creatinine clearance is independent of diuresis, and urea clearance is not. Further the variation of the urea clearance with diuresis is not yet fully cleared up. The equation for the standard clearance, which in itself represents a serious obstacle for so many, is far from satisfactory both from a practical and a scientific point of view. A standard clearance is in reality nothing more than a mathematical

trick, which is performed in order to avoid throwing away blood and urine specimens when the diuresis happens to lie below 2 ml per minute.

Further, urea clearance is to a significant extent dependent on protein consumption. It may also show quite unexplainable variations of surprising extent..

A correct appreciation of urea clearance requires quite a bit of insight, at least if the test is to give real information of diagnostic or prognostic value. Finally it may be said to the advantage of the "creatinine" clearance test that it is much easier to perform than the urea clearance test.

The second aim of this paper is to establish the level of *endogenous "creatinine" clearance in relation to the glomerular filtration rate*. In normal individuals the average endogenous "creatinine" clearance was 16 per cent lower than inulin clearance. In cardiac patients the difference was 11 per cent, measured partly in relation to inulin clearance, partly to thiosulfate clearance. This comparison applies to a wide range of glomerular filtration rates, as the experimental procedure sometimes brought the inulin clearance down to 40 ml per minute. The good correlation between endogenous "creatinine" clearance and inulin clearance, and between endogenous "creatinine" clearance and thiosulfate clearance will be apparent from Fig. 5, 6 and 7, and has also been established by mathemathical analysis.

Regarding the ratio endogenous "creatinine" clearance/inulin clearance, a statistical analysis shows that the difference of the coefficient of regression between normals and cardiac patients is not significant. For all

these individuals the coefficient of correlation is 0.76 and the coefficient of regression is 0.99. From a known "creatinine" clearance the corresponding inulin clearance can be estimated according to the equation: Inulin clearance $= 17.21 + 0.99 \times$ creatinine clearance, i.e. for practical use: Inulin clearance $= 17 +$ creatinine clearance.

That the endogenous "creatinine" clearance lies 11—16 per cent lower than the glomerular filtration rate tallies well with the fact that the "creatinine" concentration we determine in plasma lies from 0 to 20 per cent above the real concentration (Miller and Dubos 1937, Allinson 1945). Our results therefore support the hypothesis that the real endogenous creatinine clearance represents the true glomerular filtration rate (Miller and Winkler 1938). With our technique the deviation from 1.00 in the ratio endogenous "creatinine" clearance/inulin clearance is statistically fully significant. But with the same technique Brod and Sirota (1948) found the same ratio exactly 1.00. This result, which is somewhat unexpected, is not discussed by the authors. We are not able to explain the difference between their results and ours.

In conclusion it may be said that the endogenous "creatinine" test performed as in these investigations is a very good index of the glomerular filtration rate although not quite equal to that value. A practical method which determines only the real creatinine in plasma would be a step forward.

CONCLUSION

The average normal endogenous "creatinine" clearance is 105 ml per minute $\pm$ 21 per 1.73 m² surface. The endogenous "crea-

tinine" clearance is in normals and in cardiac patients about 60 per cent higher than urea clearance. In renal disease the endogenous "creatinine" clearance is almost twice the urea clearance. There is good correlation between these two values. For practical purposes endogenous "creatinine" clearance has considerable advantages, and the test is recommended for practical use in the clinical laboratory. It is simple to perform and easier to evaluate than the urea clearance test. The level of the plasma concentration of endogenous "creatinine" will give the clinician a fairly good idea of the extent of the kidney lesion.

In normals and in cardiac patients there is a good correlation between endogenous "creatinine' clearance and inulin or sodium thiosulfate clearance. The average difference is in normals 16 per cent, in cardiac patients 11 per cent.

The results establish more firmly the conception that the real endogenous creatinine clearance is identical with the glomerular filtration rate.

SUMMARY

Simultaneous determinations of endogenous "creatinine" clearance and urea clearance have been performed in 40 normal individuals (51 clearance periods), in 30 patients with valvular heart disease (54 periods) and in 36 patients with renal disease (36 periods). Simultaneous determinations of endogenous "creatinine" clearance and inulin clearance have been performed in 37 normal individuals (103 periods) and in 27 patients with valvular heart disease (100 periods). In 13 cardiac patients and 2 normals endogenous "creatinine" clearance has been performed simultaneously with sodium thiosulfate clearance (85 periods). The results are discussed with regard to the practical use of endogenous "creatinine" clearance instead of urea clearance and further as an index of the glomerular filtration rate.

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ENDOGENOUS FORMATION OF CARBON MONOXIDE IN MAN UNDER NORMAL AND PATHOLOGICAL CONDITIONS

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When determining the carboxyhemoglobin concentration by analysing the CO concentration of the alveolar air according to a method described earlier (Sjöstrand, 1944, 1948), it has been observed that a low concentration of a substance, oxidized in the same manner as carbon monoxide in the CO meter used, is present invariably in the alveolar air in man. At first it was assumed to be due to carbon monoxide absorbed from the atmospheric air, but when it was discovered that the alveolar CO concentration may vary very considerably under pathological conditions, the observation has been subjected to closer analysis. The results seem to show that carbon monoxide is constantly formed in man in small quantities, and under certain pathological conditions this formation may be considerably increased.

METHOD OF PROCEDURE

The alveolar carbon monoxide concentration was determined according to Sjöstrand (1944, 1948) and tests were taken in 100 % oxygen gas after expiring the nitrogen from the lungs during 5 minutes. The majority of the determinations were carried out as blank samples in the course of subsequent total hemoglobin determinations obtained by rebreathing for 15 minutes in a system with a carbon dioxide filter (see Sjöstrand 1948 b). In addition tests were made with a smaller system consisting of a respiration valve, rubber tubes, a carbon dioxide

filter and a seven liter rubber bag. In this smaller apparatus the nitrogen was expelled from the lungs by inhalation of 100 % oxygen. The rubber bag was then filled with expiration air, and rebreathing continued for 6 minutes. By carrying out tests in this manner a value of CO concentration is found which corresponds to 90 % of that yielded by 15 minute tests in the greater apparatus. The accuracy of the determination increases with the time for rebreathing, though naturally the shorter time and the smaller apparatus is preferable for practical reasons. The test error in the latter case is approximately ± 5 %.

The carbon monoxide determination in Lindelöv-Sjöstrand's CO meter must be carried out with great accuracy when determining the low carbon monoxide concentrations, with which we are concerned here. Repeated determinations of a calibrating gas mixture should be carried out and it is particularly important to see that the apparatus is steady before the test is determined. If the determinations are made in this manner, the carbon monoxide concentrations here concerned, about 0.002 %, can be determined with an accuracy of ± 3 %. The lower limit for estimation with a standard apparatus lies at 0.0001 % CO. With special sensitive CO meters a concentration of 0.00002 % can be estimated.

PROOFS OF AN ENDOGENOUS CARBON MONOXIDE FORMATION

In the introduction to this paper it was pointed out that a substance oxidized in the same manner as carbon monoxide in the CO meter was found constantly in the alve-

olar air in all the subjects so far examined, the number being approximately 1000 men, women and children. A considerable number of these people were tobacco-smokers or might have been exposed to a low concentration of carbon monoxide in their work. However, even in non-smokers and in those who could not be considered as being exposed to carbon monoxide it was possible to show an alveolar carbon monoxide concentration of about 0.002 % when a test was taken with 100 % oxygen. In order to analyse this observation it was necessary: 1) to endeavour to determine wether it really is carbon monoxide that gives this indicating effect and 2) to endeavour to show that the presence of this factor in the alveolar air is not due to carbon monoxide being absorbed from the atmospheric air.

· The CO meter used is constructed on the principle that carbon monoxide is oxidized by a metallic oxide mixture (hopcalite) at room temperature, whereupon the heat effect is measured on a differential thermometer. This reaction is not quite specific and might be caused by other easily oxidizable substances. In order to show that this is a carbon monoxide effect a comparison has been made between the CO-meter method and two other methods for CO estimation in air. In the one method the combustion effect of hopcalite at room temperature was used, and the consequent CO_2 production was estimated by absorbing CO_2 in $Ba(OH)_2$ solution or Ascarite. The precipitate of $BaCO_3$ obtained in the former case was dissolved in hydrocloric acid after washing, whereupon Ba was precipitated with sulphuric acid and $BaSO_4$ was determined gravimetrically. In using Ascarite the weight

of the filter was determined before and after the air passage.

The third method used was the iodine pentoxide method, based on the fact that at 140° C carbon monoxide is oxidized by iodine-pentoxide with the liberation of iodine. This is absorbed in a solution of potassium iodine and determined by titration with thiosulphate (see Eiseman 1945).

In Table I a comparison is made between the determinations on alveolar air samples with the three methods. In using the CO meter and the hopcalite-CO_2 methods, the determinations have been calculated by comparison with CO-gasmixture of known CO

T a b l e I. *Determination with three different methods of the CO concentration in alveolar air samples obtained by rebreathing oxygen.*

Sample No.	CO-meter value	Combustion and determination of CO_2	Iodine pentoxide method
1	0,0026	0,0024	0,0024
2	0,0012	0,0013	0,0015
3	0,0019	0,0022	0,0016
4	0,0015	0,0020	0,0015
Mean	0.0018	0,0020	0,0018

concentrations. The results show that the three methods give approximately the same values of the CO-concentrations which strongly supports the supposition that the active substance is carbon monoxide.

This statement was also supported when a comparison was made between the "CO" concentration in alveolar air samples when breathing atmospheric air and 100 % oxygen. The CO concentration by breathing oxygen and air respectively showed a difference of

Table II. *Comparison between the CO concentrations by breathing air and 100 % oxygen.*

Test person	CO in exspired air in %	CO% by re-brathing 10 times deeply* or during 2 minutes	CO% in ex-spired air by breathing 100% oxygen	CO% by re-breathing 10 times deeply* or during 2 min. of 100% oxygen	CO% by re-breating 100% oxygen 6 min.
K-l 27.4	0,00017	0,00025	0,00045	0,00103	0,00152
K-l 28.4	0,00013	0,00026	0,00064	0,00097	0,0018**
H-d	0,00013	0,00025*	0,00068	0,0009*	0,0017
E-m		0,00033*	0,00098	0,0010*	0,0032

* Mean value of a large number of determinations at different days.

le same ratio as when carbon monoxide is)erated from the blood i.e. about seven times reater concentration by using oxygen than :r when an equilibrium has been established Table II). Due probably to the circulatory ictor of the CO elimination there are smal- :r differences between the expiratory air :sts and shorter rebreathing tests in oxygen espectively air.

In order to endeavour to decide whether he carbon monoxide concentration in the lveolar air was due to absorption from the .tmosphere or to endogenous formation a letermination of the CO concentration of the .lveolar air was carried out both before and .fter 5 or 6 hour's breathing through a CO ilter. In a preliminary experiment this pro- :edure appeared to completely eliminate the :O concentrations in question. In one :xperiment the alveolar CO concentration vas reduced to one third by breathing 100 %)xygen gas in an open system before the)reathing was undertaken through the CO ilter. As will be seen in Table III, the alve-)lar carbon monoxide concentration did not :ink while respiration was going on through he CO filter, on the contrary, it rather rose :omewhat. If the carbon monoxide in the ilveolar air owes its precence to an absorp-

Table III. *Alveolar CO concentration before and after breathing through a CO filter.*

Testperson	Time for the test in hours	Alv. CO in per cent be-fore breath. through filter	Alv. CO in per cent after breathing through filter
J-n	5	0,0013	0,0015
H-d	6	0,0024	0,0040

tion of carbon monoxide in the blood from outside air, the alveolar carbon monoxide concentration should be reduced to about 40 % after 5 hour's respiration of carbon monoxide. free air.

The fact that the alveolar carbon monoxide indicated originates from the blood is proved by the statement that when a test is taken with 100 % oxygen or when breathing 100 % oxygen in an open system the elimination quantitively corresponds to a CO quantity in the body of the same magnitude as can be calculated from the COHb concentration and the total quantity of hemoglobin. It has also been made clear that carbon monoxide is constantly being emitted from the lungs from the fact that when alveolar air samples are taken with air a low CO concentration can be shown. The particularly sensitive CO

T a b l e IV. *Determination of the CO formation in the body.*

Test pers.	Tot. Hb. Gram	Time for determin- ations	Before the determin- ation period		After the determin- ation period		Expired vol. CO ml.	Vol. CO in ml. formed pro hour
			COHb %	Tot. vol. CO in the blood ml.	COHb %	Tot. vol. CO in the blood ml.		
K-1	450	3	0,41	2,5	0,34	2,1	1,9	0,50
H-d	550	2 1/2	0,37	2,75	0,37	2,75	1,7	0,70
Å-m	440	3 1/4	0,34	2,05	0,30	1,80	3,4	1.00

meter also made it possible to show small quantities of carbon monoxide in the expiratory air over and above the possible CO percentage of the inhaled air during normal breathing of atmospheric air. Estimations of the amount of CO exhaled during 2.5 or 3 hours was also made, by collecting the expiratory air in 200 liters bags during repeated 30 minute periods. Table IV shows that on three different test persons 0.5, 0.65 resp. 1.0 ml CO was exhaled per hour, with little or no change of the COHb concentrations during the test period. It is also a routine experience in the investigation of laboratory subjects and hospital patients to find that the alveolar CO concentration is very constant and that CO invariably occurs in the expired air despite the fact that the inspired air does not contain CO concentrations measurable with the most sensitive CO meters.

Hence it follows that the alveolar carbon monoxide originates from the blood and that small quantities of carbon monoxide are constantly emitted with the air exhaled, being replaced by carbon monoxide produced within the body. Since this alveolar carbon monoxide concentration is fairly constant in the same individual, it must be presumed that there is an equilibrium between the CO formation in the body and the elimination through the lungs. If this equilibrium is upset, e. g. by the respiration of oxygen, then the COHb concentration of the blood will fall, until a new state of equilibrium is reached between the CO formation, the alveolar CO partial pressure and the CO elimination with the respiratory air.

NORMAL VARIATIONS OF THE ALVEOLAR CARBON MONOXIDE CONCENTRATION

In normal circumstances and when tests are taken under similar conditions the alveolar carbon monoxide concentration is fairly constant in the same individual, as will be seen in Table V, and Fig. 1.

T a b l e V. *Mean ± standard error of the mean by repeated determinations of the CoHb concentrations on three test persons not exposed for Co.*

Test person	Number of determinations	COHb per cent
B-r	15	0,48 ± 0,032
K-1	32	0,51 ± 0,018
H-d	15	0,65 ± 0,025

These determinations in the table have been obtained from three assistants of the female staff at the laboratory and have been going on for more than a year. The seasons do not seem to produce any definite varia-
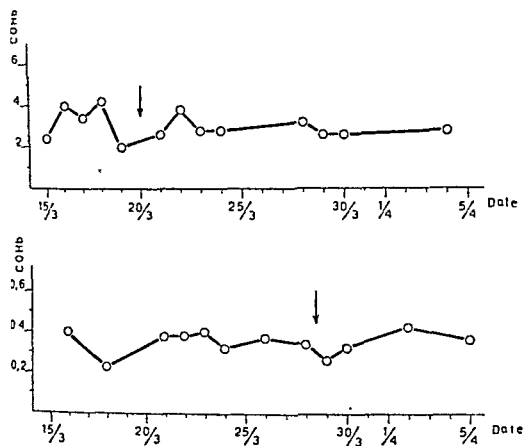
Fig. 1. The variation of the calculated COHb concentrations on two female individuals during a 3 weeks period. ↓ = menstruation.

ated from the alveolar carbon monoxide concentration in children shows fairly good correlation to body weight, though such is not the case with adults, which show a better correlation with the hemoglobin quantity. Children also show somewhat higher values than adults, and men apparently have somewhat higher values than women.

The values of the alveolar carbon monoxide concentration that were found when tests were taken with oxygen naturally do not give directly comparable values of the endogenous carbon monoxide formation, since the total hemoglobin quantity as well as the respiration influence the alveolar CO values. Since a comparatively large quantity of carbon monoxide is eliminated every time when tests are taken with oxygen, the COHb concentration of the blood will sink during the test the smaller the total carbon monoxide content of the blood. Nevertheless this cannot altogether explain the correlation that seems to exist between the alveolar CO values and the total hemoglobin quantity. The average respiration of men, which is greater as compared with that of children and women, must lead to a more rapid elimination of the carbon monoxide from the blood, in consequence of which the endogenous CO production on males may be expected to be greater than on children and women.

tions though it seems possible to trace a variation in connection with menstruation, the alveolar carbon monoxide concentration falling somewhat in connection with the menstrual bleedings.

This constancy of the alveolar CO concentration has also appeared in duplicate determinations on 40 individuals, when there was a variation of $\pm$ 13 % including the error of determination in the method.

The values from different individuals are also of the same magnitude on the whole. Table VI shows the variability between children, women and men, who as far as can be ascertained, have not been exposed to carbon monoxide in any way. From the table it will also be seen that COHb calcul-

Table VI. *The CoHb concentrations on different persons not exposed to Co.*

Material	Number of observ	COHb%	Correlations to		
			body weight	body surface	tot. Hb.
Children	57	0,54 ± 0,02	+ 0,64	+ 0,59	+ 0,53
Women	36	0,46 ± 0,016	+ 0,27	+ 0,24	+ 0,46
Men	46	0,51 ± 0,012	± 0,39	+ 0,36	+ 0,49

Table VII. *Relative Hb, total Hb and CoHb in cases of polycythemia.*

Subj	Sex	Rel Hb%	A Total Hb calcul. grams	B Total Hb obtained grams	Diff. A-B in % of A	COHb%	Notes
O—n	male	110	745	1150	+ 55	0,67	
B—e	male	140	885	2350	+ 165	0,84	
W—e	male	115	690	1690	+ 145	0,73	
K—s	male	140	785	2000	+ 135	0,61	
K—n	female	145	630	1040	+ 65	0,84	
C—m	female	120	500	1090	+ 118	0,63	
Ö—m	female	140	405	1240	+ 206	0,65	
U—g	female	115	435	930	+ 114	0,44	
R—n	female	165	485	1055	+ 117	1,13	
		152	500	815	+ 63	0,84	2 month aft. P³¹
		84	515	485	— 6	0,71	9 " " "
E—n	female	115	610	1270	+ 108	0,59	
A—n	female	135	460	980	+ 113	0,76	

PATHOLOGICAL VARIATIONS OF THE ALVEOLAR CARBON MONOXIDE CONCENTRATION

The alveolar carbon monoxide concentrations show very considerable variations under pathological conditions. In certain diseases a striking increase has been noticed in the concentration.

A leg fracture involving long decumbiture

In a case of fracture of tibia et fibula which was set without any shedding of blood, and which was admitted to the hospital for nearly 2½ months, COHb concentrations calculated of the alveolar CO values as well as the total hemoglobin quantities were followed with repeated determinations. In Fig. 2 it will be seen how both the COHb values and the total hemoglobin quantities sank during the decumbiture. When the patient began to get up again, there was a manifest decrease in the COHb values, which in over 2000 determinations reached the lowest hitherto recorded. Afterwards there was a rise both in the CO and in the total hemoglobin values.
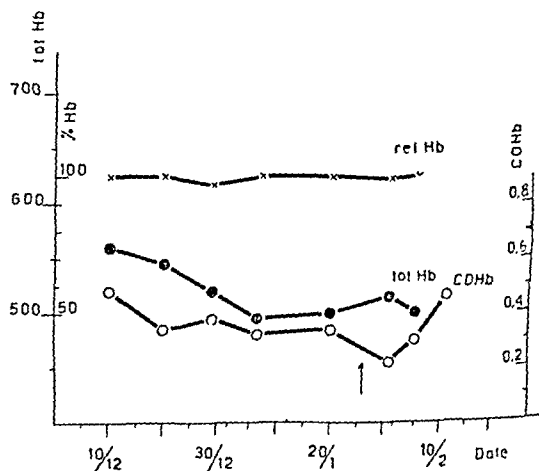
Fig. 2. The relative hemoglobin values, the hemoglobin weights (in gram) and carboxyhemoglobin concentrations during and after bedrest on a patient with bone fracture. At ↑ the patient is allowed to leave the bed for the first time.

Polycythemia

From Table VII it will be seen that higher COHb concentrations have been observed in cases of polycythemia than is usual in normal material. This increase corresponds to a great extent to the relation found between the alveolar CO values and the total hemo-
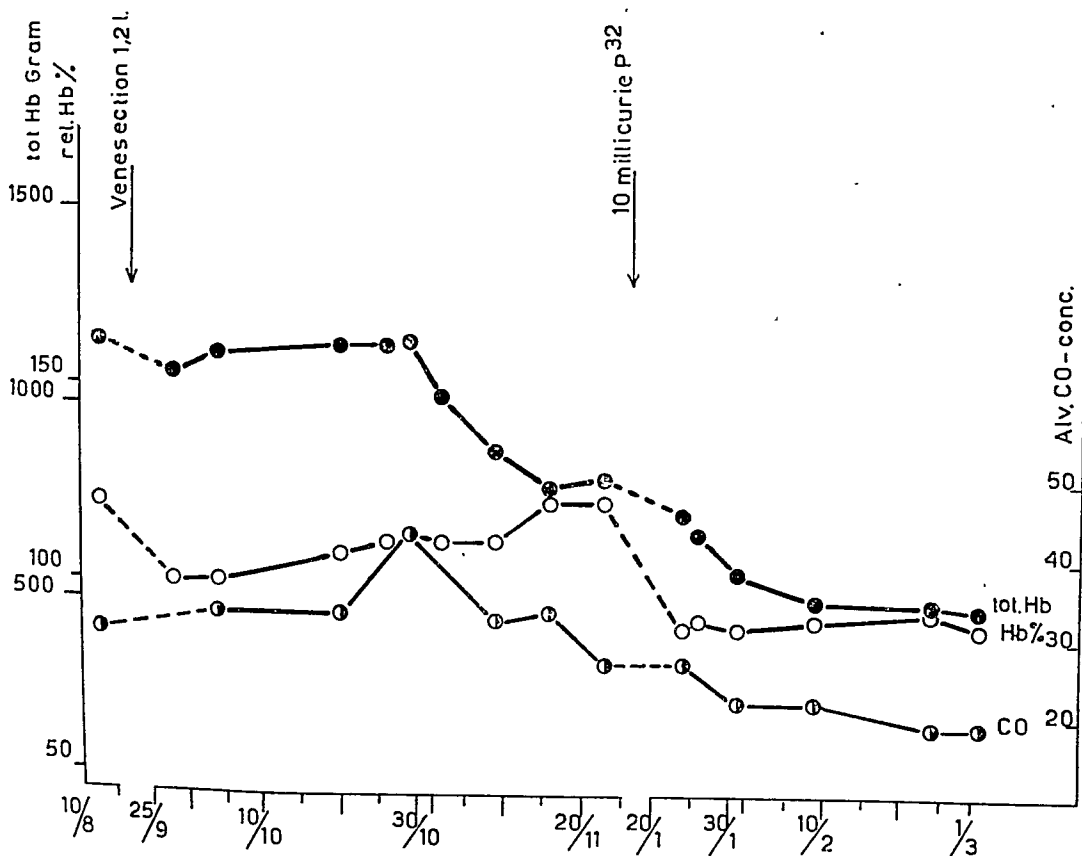
Fig. 3. The total amounts of hemoglobin, the relative hemoglobin values and the alveolar CO concentrations by breathing oxygen on a case of polycythemia, before and after treatment with radioactive phosphorous.

globin quantity in normal adults, but in other cases the increase has been somewhat greater. The table as well as Fig. 3 also shows that there is a reduction in the CO concentration when there is a decrease in the total hemoglobin quantity in connections with treatment with radioactive phosphorus. Fig. 3 also seems to show that a spontaneous regression is followed by a decrease. It has not been possible to decide from the material concerning polycythemia hitherto studied whether the increase observed prior to the spontaneous regression is significant or not.

Pernicious anemia

Three cases of pernicious anemia have so far been studied in this respect, and two of them have shown that the alveolar CO-concentrations are unusually high at the beginning of a specific therapy but gradually become normal. This will be seen in Fig. 4, where the calculated COHb values in one of these cases as well as the Haldane values and total hemoglobin quantities in per cent of calculated normal value are correlated to the time allotted for treatment. In the second case the initially determined values
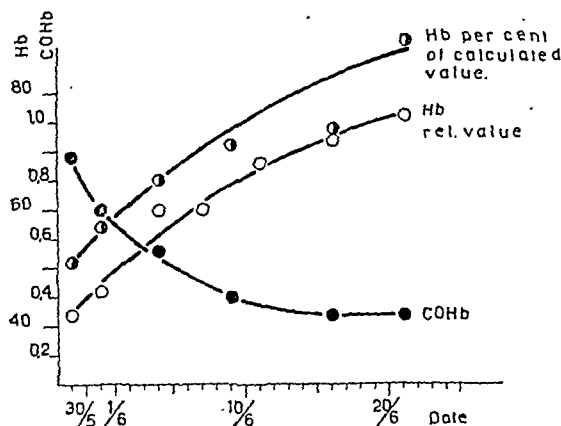
Fg. 4. The total hemoglobin in per cent of from body weight calculated value, Haldane values and the COHb concentration on a woman with pernicious anemia. treated with specific therapy.

were still higher, but in this case three blood transfusions had been given during the first few days, which, as will later be shown, may in itself cause a considerable increase in the alveolar CO concentration. However, even two weeks after these transfusions the CO value was about twice as high as when the full effect of treatment had been achieved, which corresponded to the normal values.

Aplastic anemia

The determination of the alveolar CO concentration has been carried out in three cases of aplastic anemia, one of which showed signs of an increase in the break down of the blood, inter alia the colour of the skin was slightly icteric. This case was under hospital observation both during 1948 and 1949, and on both occasions the values were about double the normal. Determinations were also taken the day after blood transfusion on five occasions, when the CO concentration showed a further rise to 0.006 % i. e. three times the normal value. The other two

aplastic anemias showed no signs of increased blood destruction, they had also normal CO concentration in the alveolar air.

Anemia in connection with acute exacerbation in a case of chronic nephritis

Fig. 5 illustrates the relation of the total hemoglobin quantity, the calculated COHb concentration and the body temperature in a case of nephritis during a state of acute exacerbation. It will be seen that during a first period of three weeks, when comparatively frequent blood transfusions were administered, the hemoglobin values did not increase except in direct connection with the blood transfusions, but afterwards there was a continual increase. The COHb values are more than double the normal value during this period and increase with each transfusion. During the latter part of the disease the alveolar values are normal except for the day after one transfusion. The tempera-
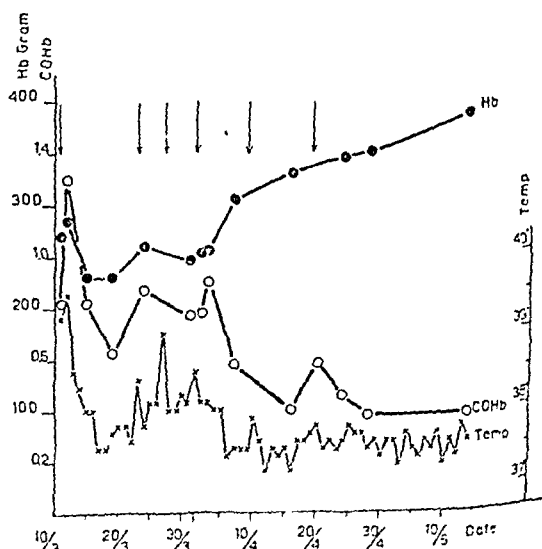
Fig. 5. The total amount of hemoglobin, the COHb concentration and the body temperature of a case of acute exacerbation of nephrites ↓ = blood transfusions.
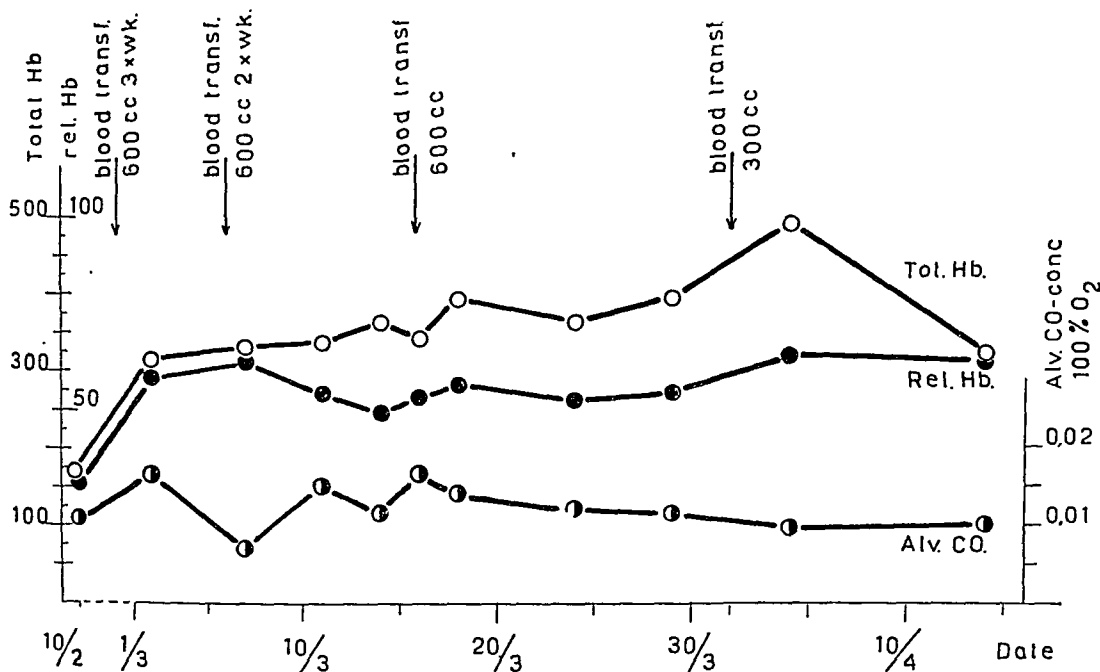
Fig. 6. The weight of hemoglobin the relative hemoglobin values and the alveolar CO-concentra-
tions by breathing 100 % oxygen of a case of hemolytic anemia. The CO-curve lies 5 to 7
times higher than normal during the observations period.

ture curve largely resembles that of the COHb curve. Hence, after most trans-fusions the body temperature rises for 12—14 hours, and particularly in cases of trans-fusions when the COHb values are the highest.

Hemolytic anemia

A case of hemolytic anemia made its appearance at the beginning of a pregnancy and was followed for two months. This case shows the highest alveolar CO values that have hitherto been recorded from indivi-duals not exposed to carbon monoxide. The highest values were 0.015 % when the test was taken with 100 % oxygen gas and 0.002 % with air. These values correspond to a COHb concentration of 2.8 %. During

the time of observation these values increased slightly at first, to sink somewhat at the end of the observation period in connection with a small rise in the total quantity of hemo-globin and the relative Hb value. The lowest value hitherto recorded in this case is 0.009 % when the test was taken with oxygen. So far about 100 determinations have been carried out in this case, and it has been possible to avoid any exposure to CO. The CO_2 analysis of the alveolar gas sample after combustion in the CO meter, as in-dicated above, proves that the combustible material is carbon monoxide. Fig. 6 illu-strates the relation between the alveolar CO values, the total hemoglobin values and the relative Hb values during the time of ob-servation.
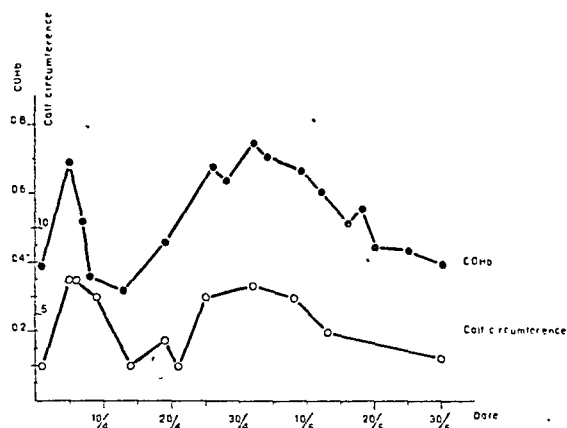
Fig. 7. The COHb-concentration and the circumference of the one calf on a man with veinous thrombosis. After a short increase of the measures at the beginning of the observation periode, there is a decrease during anticoagulant therapy. The dosage of the anticoagulant is decreased after about a week and the calf circumference and COHb-values increase for some time.
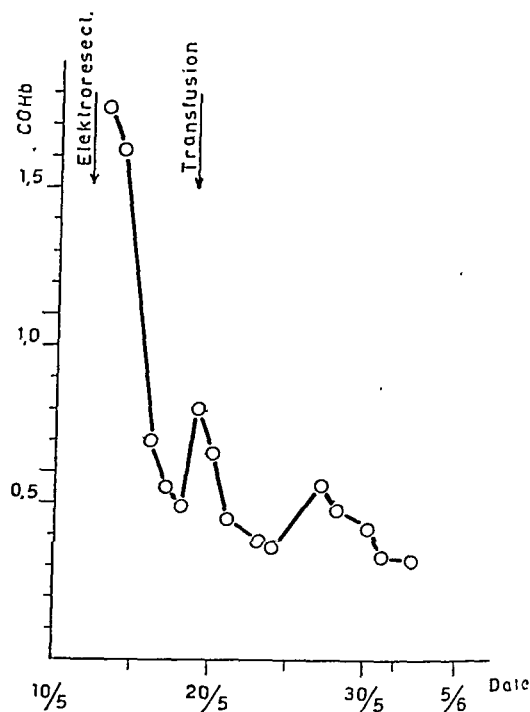
Fig. 9. The COHb-concentration after transurethral prostatic resection with very marked hemolysis of the blood. The first two days after the operation and in accordance to a blood transfusion there is an increase of the COHb-concentration.

Fig. 8. The CO-concentration of the alveolar air by breathing 100 % oxygen during the treatment of varicose veins by provoking artificial thrombosis with etolein.

Vein thrombosis

Fig. 7 shows the relation between the COHb-concentration and the circumference of the calf in a case of vein thrombosis. After an acute stage with increase of both the thickness of the calf and the COHb concentration, there is a decrease following anticoagulant treatment. After some days the dosage of the anticoagulant (dicoumarine) is decreased and the COHb-concentration increases as well as the circumference of the calf. Consequently there seems to be an intimate correlation between the development of the circulatory disturbance in the leg and the COHb-concentration.

An increase of the COHb-concentration in veinous thrombosis has also been found in two other cases. The concentration seems also to decrease very rapidly after anticoagulant treatment. In seven cases of artificial thrombosis following treatment for varicose veins a similar increase of the alveolar CO-concentration was observed. One of the cases is demonstrated in Fig. 8. In one other case a definite increase of the COHb did not occur after the treatment, which may be attributed to less successful therapy or to the fact that the COHb-reaction is not constant in these cases.

Hemolysis in Connection with Transurethral Prostatic Resection

In cases of transurethral prostatic resection hemolysis sometimes occurs following the entrance of water into opened blood vessels. Two such cases, followed by CO determination of the alveolar air, showed an increase of the CO-concentration in direct connection with the operation. The hemolysis was also confirmed by direct observation of free hemoglobin in serum. In the one case, demonstrated in Fig. 9, it was also seen that an increase of the COHb-concentration occurred after a blood transfusion.

Blood Transfusions in Cases of Secondary Anemia After an Acute Ulcus Bleeding

The alveolar CO concentration has been determined in three cases of secondary anemia following bleedings from a duodenal wound. One of these cases showed increased values after blood transfusions (Fig. 10), but the remaining two, which did not get any blood transfusions revealed normal values. The case illustrated in Fig. 10 pre-

sented a temperature curve with only a slight rise when the first transfusion was carried out. The rise in the CO values in the case of blood transfusiones is demonstrable 6 hours and sometimes up to 72 hours after the transfusion. An increase of the alveolar CO concentration in connection with blood transfusion have been demonstrated in a total of 10 different cases and on some cases several times.

The increase after the operation in Fig. 10 is probably also significant. The same has been found the day after surgical operations in three other cases.

Pathological Conditions in Which Normal Alveolar CO Values have been Recorded

So far cases of the following diseases have been examined without showing any rise in the alveolar CO values: secondary anemia with sideropenia and after acute bleedings, various types of heart diseases, also incompensated heart failor, hypertension, Basedow's disease, diabetes mellitus, colitis ulcerosa, polyarthritis, hepatitis, myelogenic leukemia, cancers of the breast, urinary bladder, lungs, larynx and stomach, papilloma of the bladder, and bronchial asthma.

DISCUSSION

Gréhant (1894) seems to be the first one, who has found small amounts of a combustible gas in the blood. The observations of de Saint-Martin (1898) seem to indicate for the first time that this phenomenon is probably due to the presence of carbon monoxide. Several other French authors have made further observations on this point especially on dogs. Nicloux (1898, 1901, 1902, 1928) tried to show that carbon mo-
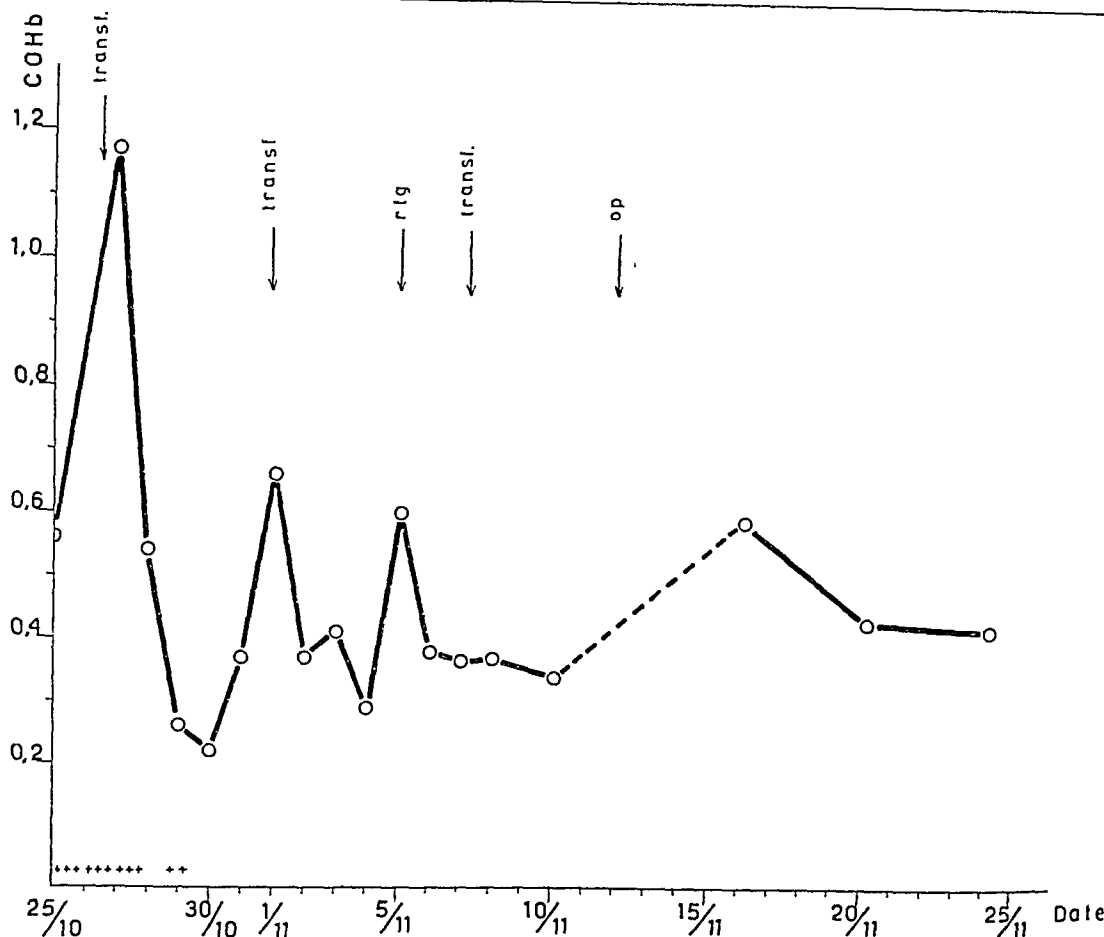
Fig. 10. The carboxyhemoglobin concentration on a case with acute bleeding from a duodenal ulcer. After the blood transfusions the COHb-concentration increase. $++$ = blood in feces.

noxide is present in the blood due to endogenous formation. Nicloux and Nebenzahl (1929) concluded that it is produced during the prosesses of sugar metabolism in the body. Rathérey, Gley, Franc and Goursat (1932) claim to have found evidence of CO production in the central nervous system and Lepine and Boulud (1905) found carbon monoxide in the blood on post mortem examination of some cases of anemias. These authors are of the opinion that carbon mo-

noxide production has some not very clearly defined connection with oxalic acid metabolism (1906).

Loeper, Bioy, Gilbrin and Tonnet (1936) and Loeper and Tonnet (1940) claim to have found an increase of the CO in the blood on patients with anoxia of different causes and Loeper (1939) speaks about a CO intoxication due to an endogenous formation. Jongbloed (1939) found traces of a gas, which seemed to be CO in alveolar

air in nonsmokers. Roughton and Root (1945) found by estimation of the blood volume in man with the CO method that blank samples of blood contained small amounts of carbon monoxide but could not confirm the observation of Rathéry, Gley, Franc and Goursat (1932) that the blood in the jugular wein contains higher concentrations of CO than the arterial blood.

Several authors have, consequently, found a gas which probably is carbon monoxide in the blood on human beings and dogs or in alveolar air samples The origin of the CO found, if it is the result of an uptake from the atmosphere or of a production of CO in the body, has, however, not been definitely shown. Some of the communications mentioned, especially those which deal with the question of the endogenous CO formation, are also very little convincing. The observations described here seem, however, to show that the gas is carbon monoxide and definitely also that it is continually formed in small amounts under normal conditions in man.

In normal circumstances this endogenous CO formation seems to be of the same magnitude in different individuals, though in pathological conditions it may be considerably increased. Judging from observations so far made, it seems as though the alveolar CO concentration can increase up to seven times the normal value. The pathological cases with increased alveolar CO values so far observed indicate that there is a rise in the endogenous CO formation in cases with increased blood cell destruction. This explains the higher values found in polycythemia, where an increased production as well as break down of blood cells must be assumed,

in some cases of pernicious anemia, aplastic anemia when an increased destruction of blood cells is apparent, above all in hemolytic anemia, and in hemolysis due to transurethral prostatic resection, blood transfusions and veinous thrombosis. The determinations hitherto carried out in pathological cases are not sufficiently numerous to justify a decision of wether it is only in these and similar conditions that the endogenous CO formation is increased, but determinations on more than a hundred different pathological cases seem to show that an increase indicates increased decomposition of blood. This reaction seems also to be very sensitive and it seems possible, consequently, that it can be used for diagnostic purposes.

SUMMARY

With a method described earlier (Sjöstrand 1944, 1948) carbon monoxide in a concentration of 0.0015 %—0.003 % can be constantly shown in alveolar air analysis during inhalation of 100 % oxygen gas. Even in experiments made during the respiration of ordinary air it is possible to show a carbon monoxide concentration of about 0.0003 %. When breathing atmospheric air it has even been possible to show corresponding concentrations of about O.00013% in the expiratory air.

This presence of carbon monoxide can also be shown on people resting for several hours and days in air not containing a corresponding carbon monoxide concentration or breathing through a CO-filter during 5—6 hours, without a decrease of the alveolar carbon monoxide concentration.

The amount of carbon monoxide expired during 2½—3 hours was also determined

and was found — after correction for eventual change of the COHb concentration — to be 0.5, 0.65 and 1.0 ml pro hour in three test persons.

These observations seem to show that carbon monoxide is constantly being formed in the human body.

The alveolar carbon monoxide concentration is fairly similar in different normal individuals, although it shows a ' certain correlation to bodyweight during the growing age and to the total hemoglobin quantity in adults.

In pathological conditions the alveolar carbon monoxide concentration may be considerably increased and even go up to seven times the normal value, the corresponding COHb concentrations being about 3 per cent.

In the material so far investigated increases have been discovered in cases of polycythemia, two cases of pernicious anemia, aplastic anemia, with signs of hemolysis, hemolytic anemias, in connection with blood transfusions, hemolysis after transurethral prostatic resection and in spontaneously and artificially developed veinous thrombosis.

On the bases of these observations it appears that this endogenous CO formation is associated with the decomposition of blood cells in the organism.

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DETERMINATION OF THE TOTAL AMOUNT OF HEMOGLOBIN IN ANEMIA AND POLYCYTHEMIA

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The clinical judging of cases of anemia and polycythemia has hitherto been based only on the relative hemoglobin values. These values, however, are influenced by the plasma volume, which latter can not always be supposed to compensate the decrease in erythrocytes in anemia, or to remain constant in polycythemia. In these diseases it should therefore be of interest to complete the relative hemoglobin values with determinations of the total amount of hemoglobin.

The result of a series of determinations of the total amount of hemoglobin in a number of cases of anemia and polycythemia are reported below, chiefly with the view of demonstrating the practical value of this procedure.

METHOD

The total amount of hemoglobin was determined according to Sjöstrand (1948), that is by the administration of small amounts of carbon monoxide, and subsequent analysis of the carbon monoxide concentration in the alveolar air. The administered volume of carbon monoxide is proportionated so that the COHb concentration reaches a level about 2 % above the blank value. As the patient inhales 95 % oxygen during the determination, the oxygen physically dissolved compensates the decrease in O_2-capacity induced by the experiment. After the determination, the COHb concentration in the blood may be lowered rapidly by the administration of oxygen in an open system. Thus determinations could be made also in greatly anemic subjects, without the patient feeling any untoward effects.

In anemia, 6—7 ml of CO were generally given, whereas in polycythemia 25—40 ml had to be administered in order to allow of a sufficient exactness of the CO determination. A complete mixture in the body is not always attained in 15 minutes in cases of polycythemia, and therefore 30 minute determinations were made in some such cases. In the calculations, the earlier published corrections were used (Sjöstrand 1948).

When calculating the expected hemoglobin amount in a given individual the mean values of the hemoglobin amount in % of the body weight, published in an earlier article (Sjöstrand 1949), were used. These are for the adult woman 0.86 % and for the adult man 1.16 %. In especially fat or especially thin individuals, this per cent value should be reduced or increased respectively, as is evident from the earlier published analysis of the relation between the amount of hemoglobin and the body weight. Here one can proceed from the ideal weight as calculated from the height.

The relative hemoglobin values were determined in finger "capillary" blood, and with the Sicca hemometer. 100 % corresponds to 15.4 g of hemoglobin in 10 ml of blood. If the total amount of hemoglobin is divided by the hemoglobin per cent value, the blood volume is obtained. As the

Hb % value obtained from finger blood is not representative of the Hb concentration in the entire blood vessel system, such a determination will not be entirely accurate In this way one can, however, obtain values comparable to one another from different persons   .

RESULTS

The relationship between the total and relative hemogobin values in hemorrhagic anemia

In 2 cases of duodenal ulcer with large hemorrhages, the hemoglobin amount was determined the days immediately following the hemorrhage, and also during the restitution   One case was treated with blood transfusions, the other was not
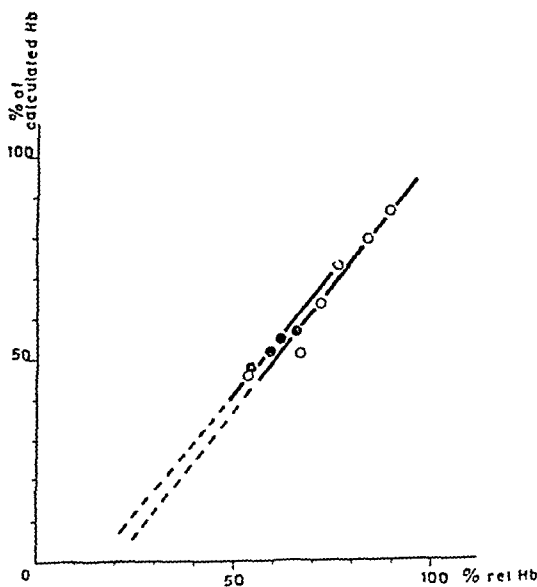
Fig 1 The relation between the estimated relative hemoglobin values and the corresponding values calculated from the determined total hemoglobin amount on two cases of hemorrhagic anemia

Fig 1 illustrates the relation between the total hemoglobin values in % of the values

calculated for each individual, and the relative Hb values (Haldane values), in the 2 cases of hemorrhagic anemia   It can be seen that the regression lines do not tend towards the zero point, which would be expected if the relative values were a direct quantitative measure of the total decrease of hemoglobin   The displacement of the regression lines shows that the plasma volume does not increase correspondingly to the loss of blood, but that the total amount of blood is smaller than before the hemorrhage   During restitution the normal relationship and blood amount are successively and parallelly reattained   At a loss of about half the hemoglobin amount, the relative Hb value was thus 62 % in one of the cases   This means that the total blood volume is nearly 20 % smaller than the normal value as calculated from the body weight, i e about 1 liter As the determinations were made during a period of 30 days, and the first values were obtained a couple of days after the hemorrhage, it is evident that the decrease in blood volume is not only an acute occurrance in connection with the hemorrhage

The repeated determinations of total hemoglobin have also possibilitated a determination of the daily production of hemoglobin above the destruction   This was in one of the cases 4 0 g (the increase caused by transfusion of course being subtracted), and in the other 5 2 g   Such values may be of interest in many respects   They are a direct quantitative measure of the blood production, and might be used for a balance calculation of the production and desintegration respectively of the hemoglobin

*The relationship between the hemoglobin amount
and the relative hemoglobin value in some cases
of chronic anemia*

Fig. 2 shows the relationship between total
Hb and relative Hb in four cases of chronic
anemia. One of these was a hemolytic
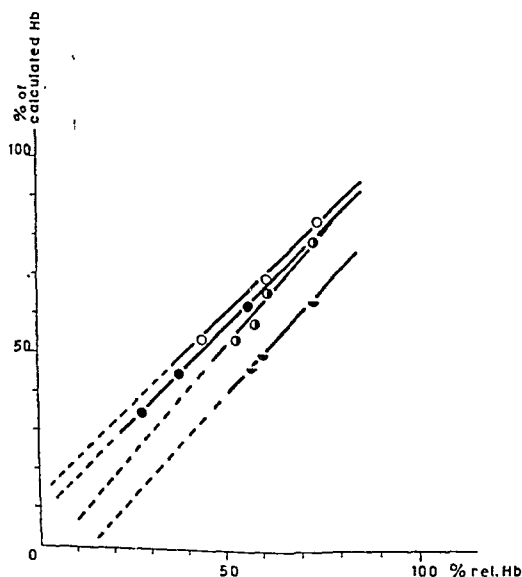
Fig. 2. The relation between relative Hb-values
and from the total hemoglobin amount calculated
values on four cases of chronic anemias. O and
●cases of pernicious anemia, ◑ hemolytic anemia,
◓ sideropenic anemia.

anemia in a case of nephritis, and another
a sideropenia. The two remaining were
cases of pernicious anemia. As is shown by
the figure these chronic anemias display a
different characteristic as compared to the
hemorrhagic anemias, in that the relative
Hb-value very nearly corresponds to the
decrease in total hemoglobin. The 2 cases of
pernicious anemia, however, present a some-
what greater total Hb-value than what could

be expected from the relative Hb-value
whereas the 2 other anemias showed a some-
what lower value.

Earlier, Rowntree and Brown (1929)
have stated that there is an increased blood
volume in pernicious anemia, a statement,
however, that is rejected by others.

The difference between the hemorrhagic
and the chronic anemias as concerns the
blood volume, explains the great difference
in their respective effect on the general con-
dition of the patient. The one case of perni-
cious anemia was up and about with a relative
Hb-value of 28 %, whereas both the hemor-
rhagic cases displayed a very poor general
condition with relative values of about 55 %.
When the blood volume in this case of per-
nicious anemia is calculated from the stated
figures, it is found to be 930 ml. i. e. 26 %
greater than the normal as calculated from
the body weight. The differences of the
blood volumes between the hemorrhagic and
the chronic anemias respectively, is of deci-
sive consequence to the adaptiveness of the
blood circulation. The symptoms of an acute
hemorrhagic anemia are chiefly caused by the
change in blood volume, and the consequent
circulatory disturbance. The diminution of
the O_2 capacity of the blood comes only
second in importance.

In order to understand the variety of symp-
toms in a case of anemia, and be able to
evaluate the circulatory adaptability e. g.
before a surgical intervention, it is thus of
importance to complete the relative hemo-
globin values with those of the total hemo-
globin amount, and to calculate the blood
volume in this way.

Table I.

Subj.	Sex	Rel.Hb %	A Hb calc-grams	B Hb opt. grams	B − A in % of A	C Bloodvol. calc.litr.	D Bloodvol. obt.litr.	D − C in % of C
O — n	male	110	745	1150	53	4,85	6,80	40
B — e	male	140	885	2350	165	5,70	10,90	91
W — e..	male	115	690	1690	145	4,50	9,55	112
K — s	male	140	785	2000	155	5,10	9,25	81
K — n	female	145	630	1040	65	4,55	4,65	2
C — m	female	120	500	1090	118	3,60	5,90	64
Ö — m	female	140	405	1240	206	2,90	5,75	98
U — g	female	115	435	930	114	3,15	5,25	67
R — n	female	165	500	1055	117	3,60	4,15	18
R — n	female	150	500	815	63	3,60	3,50	0 *
R — n	female	85	500	485	− 6	3,60	3,75	7 *
E — n	female	115	610	1270	108	4,40	7.15	62
A — n	female	135	460	980	113	3,30	4,70	42
E — d	female	140	540	1200	120	3,90	5,55	42

* After treatment with radioactive phosphorus.

The relationship between the total hemoglobin amount and the relative Hb-values in polycythemia

In Table I, the relative Hb-values, the total hemoglobin amounts. the blood volumes, and the increase of the latter as compared to the value calculated from the body weight, are collected from a number of cases of poly-cythemia. As can be seen, the total hemoglobin amounts are greater throughout, and often considerably greater than what is indicated by the relative values. The total blood volume is generally, but not always, considerably increased, the increase varying in amount from case to case.

The relative values show no simple correlation to the increase in total hemoglobin, and from a comparison in one and the same patient during treatment with radioactive phosphorus it was found that there was no simple and direct relationship between the total amount of hemoglobin and the relative Hb-values. (Se Fig. 3.)

An increase of the relative hemoglobin value of up to 110—120 % is now and then found without any increase in the total amount of hemoglobin, and it is evident that such a case is not one of authentic polycythemia. Sometimes such cases present great diagnostic difficulties, which is proved by the fact that of the cases hitherto sent to the laboratory with the diagnosis poly-cythemia, two showed no increase in the total Hb-values. In both cases, treatment with radioactive phosphorus had been instituted immediately before admission. This treatment had no effect on the hemoglobin values.

The effect of treatment of polycythemia with radioactive phosphorus can be studied in Figures 3 and 4. In Fig. 3 it can also be seen that a spontaneous regression suddenly appeared in one of the cases some time before the institution of the phosphoru-
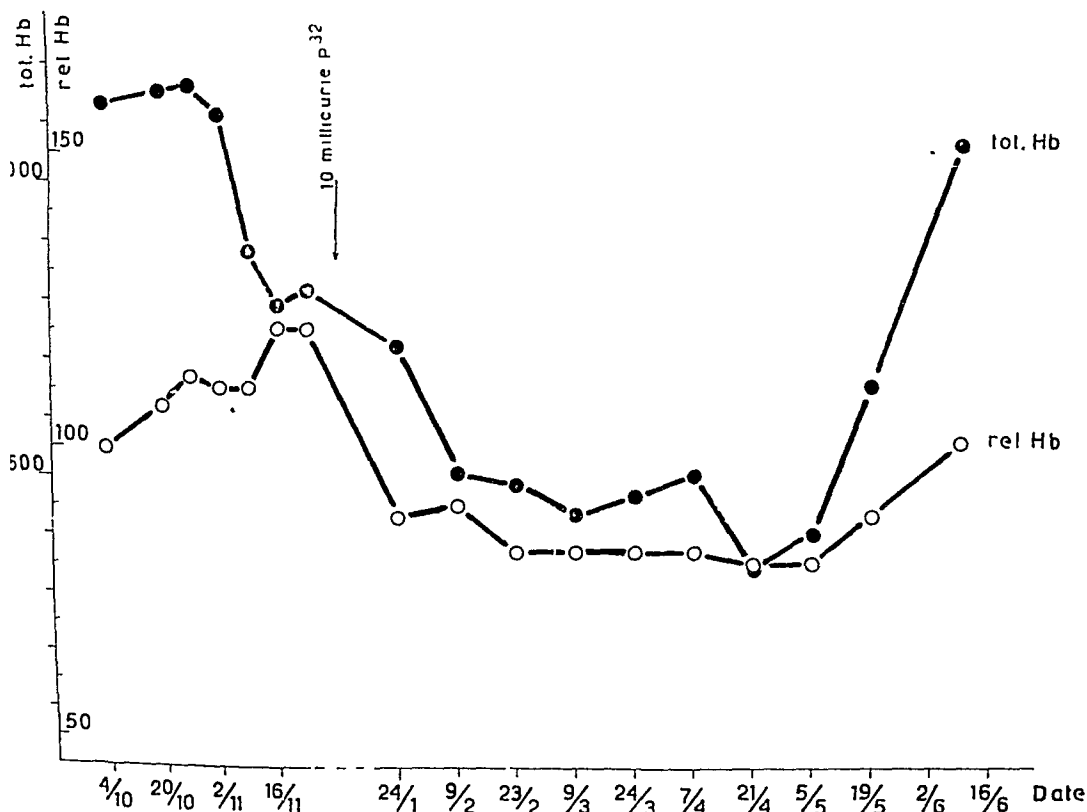
Fig. 3. The amount of hemoglobin and the relative hemoglobin values on a case of polycythemia, treated with radioactive phosphorus.

treatment. In the same patient the effect of repeated venesections was also followed, which proved very small. In this way determinations of total hemoglobin possibilitate an exact control of the effect of the treatment, and a correct estimation of the proper time for instituting treatment.

DISCUSSION

The investigation has examplified the value of determinations of the total amount of hemoglobin in cases of anemia and polycythemia. As to the anemias, the most ob-

vious advantage here seems to be that the determination possibilitates an estimation of the blood volume, and thereby the possibilities of the blood circulation to meet with different demands, perhaps especially in connection with surgical interventions.

In polycythemia the determination may be necessary for a correct diagnosis, as also for the judging of the severity of the case. Determination of the total amount of hemoglobin also makes it possible to discover spontaneous regressions, and to follow the
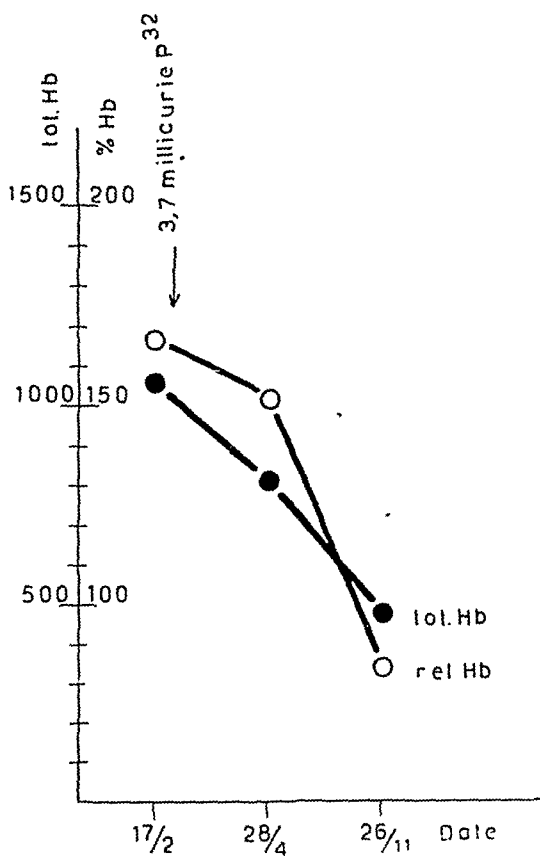
Fig. 4. The decrease of the amount of hemoglobin and the relative hemoglobin values on a case of polycythemia, treated with radioactive phosphorous.

effect of a treatment, and also to decide when it is most suitable to institute this treatment.

SUMMARY

The total amount of hemoglobin was determined by an earlier described method (Sjöstrand 1948), in two cases of hemorrhagic anemia, and four cases of different types of chronic anemias.

The hemorrhagic anemias showed higher relative Hb-values, than what could be calculated from the total hemoglobin amounts in relation to those calculated from the body weight. In the chronic group, however, two cases of pernicious anemia displayed a lower relative Hb-value. From this it could be calculated e. g. that, at the first examinations, one of the hemorrhagic cases had about 1 liter (nearly 20 %) smaller blood volume than normal, whereas one of the pernicious anemia cases had about 900 ml (26 %) greater blood volume than normal.

In the two hemorrhagic cases the amount of hemoglobin produced per day above the amount destructed was determined, and found to be 4.0 and 5.2 g respectively.

A comparison was also made between the relative Hb-values and the total amounts of hemoglobin in a number of cases of poly-cythemia.

The relative Hb-values did not correspond to the increase of the total amount of hemoglobin. Also in the individual case, the relative and total Hb-values did not always show a parallel course, e. g. during spontaneous regression or during treatment with radioactive phosphorus. The relative values generally showed a tendency to decrease later than the total values .

The chief value of determining the amount of hemoglobin in cases of anemia seems to lie in that cases of hemorrhage can thus be judged more correctly, which is especially important when a surgical operation has to be performed, and in that the restitution

can be followed quantitatively. In poly-cythemia, determination of the amount of hemoglobin can confirm an uncertain diag-nosis, possibilitate an exact judging of the severity of the case and allow of a control of the tendency of the disease and also of the result of administered treatment.

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AN ENZYMATIC METHOD FOR THE DETERMINATION OF URIC ACID BY ULTRAVIOLET SPECTROPHOTOMETRY

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In spite of the enormous work which has been done to elucidate the metabolism of uric acid, the determination of this substance still offers but little aid to the clinician. The lack of specificity of the current analytical procedures applicable to blood seems to be at least in a part responsible. Realizing this a number of authors (Blauch & Koch 1939, Bulger & Johns 1941, Buchanan, Block & Christman 1945, Block & Geib 1947, and Silverman & Gubernick (1947) have recommended different enzymatic methods based on the use of the well known colorimetric (Folin 1933, Benedict 1922) or titrimetric (Bröchner-Mortensen 1937) determinations before and after the action of a rather crude preparation of uricase. The sensitivity of these methods, however, is not particularly high and the gain of specificity, brought about by the use of the enzyme, may be reduced by the maintenance of the non-specific reduction procedures all of which require the deproteinization of the biological material and with that a most variable loss of uric acid (Bulger & Johns 1941, Jacobson 1938, Przylęcki 1928, Prætorius 1949, Pucher 1922). Moreover, in so far as these methods depend on a colorimetric procedure, they are affected by various substances which inhibit the formation of color.

In this ingenious work on the biological synthesis of purine compounds, Kalckar (1947) recently proposed to measure uric acid, the light absorption of which in the ultraviolet is very strong, by the lowering of the extinction at about 290 mμ which is caused by the addition of highly fractionated uricase to a simple dilution of blood plasma. On this principle a direct, specific, sensitive and convenient method has been elaborated for clinical laboratories which posses a spectrophotometer particularly suited to extinction readings in the ultraviolet, e. g. a Beckman spectrophotometer, DU.

The present paper deals with this method. Although elementary knowledge of the Beckman instrument and of the optical theory must be presupposed, some general headlines and a few particulars of special interest will be given. After showing the principle of the measuring of uric acid in a protein-free solution the determination as well as the recovery of uric acid in undeproteinized plasma and in urine will be demonstrated. Special circumstances in the measuring of uric acid in the spinal fluid (which are fully described elsewhere, Prætorius 1949 a) will be mentioned briefly. Finally some technical points will be discussed in detail.

METHOD

In the experiments presented below the standard quartz cells with a light path of 10 mm and containing samples of 3 ml volume are used.

In the case of *simple spectrophotometry*, i. e., when the spectrum of a compound is determined, the contents of the measuring cell differ from those of the reference cell only by the presence in the former of the compound whose spectrum is to be obtained. The extinction difference between the two cells is read directly on the transmission scale of the instrument, and is a measure of the investigated compound.*

The *enzymatic differential spectrophotometry* is the determination of the extinction change taking place in the reaction mixture of a quartz cell as a result of the enzymatic conversion of one compound into another having a distinctly different absorption spectrum. Since the difference measured is not dependent on different contents of two cells, as is the case by simple spectrophotometry, but is an extinction change taking place in the same cell, the contents of the reference cell may be chosen arbitrarily.

Reagents

Uricase. The fine white precipitate, about 3 mg, which according to the directions of Holmberg (1939) is obtained from 100 g of acetone-treated liver, is suspended in 2—4 ml of M/15 glycine buffer of pH 9.4.

Glycine buffer, 2/3 M, pH 9.4. 25 g of crystalline glycine is dissolved in about 200 ml of CO_2-free water; 110 ml of N NaOH are added and the

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* The extinction, E_λ, of an arbitrarily chosen concentration of a substance is $\log_{10} I_{c\lambda} / I_\lambda$, where I_0 and I are the intensities of light before and after absorption by the investigated substance. λ is the wave-length of the light. If Beer's law applies the concentration C of the compound is proportional to E. When a light path of 1 cm is used $E_\lambda = K_\lambda \times C$. K_λ, the specific extinction, is accordingly defined as the extinction when the concentration is 1. In this paper the unit of concentration is 1 μg per ml.

volume made up to 100 with water. The solution is saturated with chloroform (about 3 ml).

Stock solution of uric acid. A solution of lithium urate is prepared according to the directions of Folin (1933). The stability of this solution, however, cannot be ensured by the use of formaldehyde which even in the presence of a large excess of glycine produces a considerable inhibition of uricase. Therefore the urate solution is preserved by the saturation with chloroform which as no effect on the enzymatic activity.

Diluted solution of uric acid. 50 μl of the stock solution are diluted to 10 ml in a volumetric flask which after the addition of 1 ml of the glycine buffer is filled up with water. The concentration of uric in this dilution is 5 μg per ml and the molarity of the buffer M/15.

DETERMINATION OF URIC ACID IN A PROTEIN-FREE SOLUTION

The following experiment is cited in order to illustrates the agreement between the extinction value obtained by simple spectrophotometry and the extinction change produced by the enzymatic conversion of the uric acid. In this experiment the concentration of uric acid is 4.93 μg per ml.

Absorption spectrum of uric acid (simple spectrophotometry)

With M/15 glycine buffer as a reference the extinction of the uric acid dissolved in the same buffer is measured by light of various wave-lengths in the ultraviolet region. Fig. 1 shows the resulting spectrum. Abscissae: wave-length in mμ. Ordinates: the directly read extinction.

The wave-length of maximal absorption is 293 mμ. From the value of $E_{293 m\mu}$ which is 0.370, and from the known concentration of uric acid, 4.93 μg per ml, the value of $K_{293 m\mu}$ is calculated to be 0.075.

The enzymatic uric acid determination (differential spectrophotometry). Uricase suspension (10 μl) is added at zero time to the quartz cell containing the buffered uric acid solution, the spectrum of which is shown in Fig. 1. $E_{293\,m\mu}$ is read several
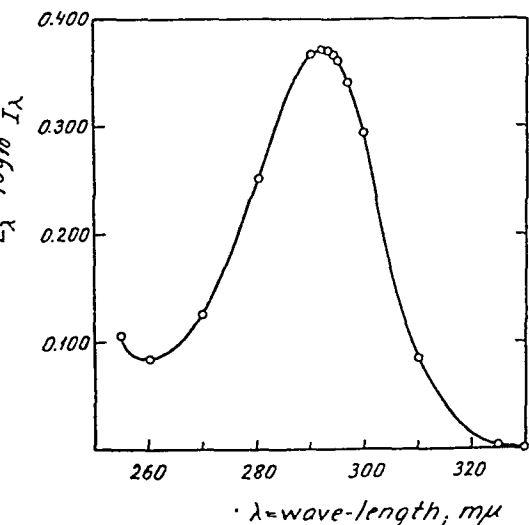
Fig. 1. The absorption spectrum of uric acid at pH 9.4. Abscissae: wave-length, mμ. Ordinates: extinction, log$_{10}$ I°/I. Uric acid concentration 4.93 μg per ml. Light path 1 cm.

times, in the beginning at short intervals; the exact time of each reading is written down. Fig. 2 shows the variation of the extinction at 293 mμ after the addition of the enzyme. Abscissae: reaction time in minutes. Ordinates: $E_{293\,m\mu}$. In the upper corner to the right the initial part of the curve is drawn on coordinates similar to those of the main curve, the only exception being the unit of abscissae which has been made considerably longer to ensure accuracy of the extrapolation to zero time.

The difference between the value of extrapolation, 0.401, and the final value, 0.031,

is 0.370. This difference, which is designated $-\Delta E_{293\,m\mu}$, was persistently found to be the same value of extinction as that observed by the simple spectrophotometry. This agreement is a confirmation of the statement of Kalckar (1947), that the absorption of uric acid at about 290 mμ completely disappears, when this compound is enzymatically oxidized. The absorption which persists when the enzymatic reaction has completed corresponds exactly to the increase of $E_{293\,m\mu}$, ($+\Delta E_{293\,m\mu}$), produced by the addition of uricases to the buffered uric acid solution i. e., the value of extrapolation minus the extinction of the uric acid equals the final value of $E_{293\,m\mu}$.

Parallel to the readings of $E_{293\,m\mu}$, $E_{340\,m\mu}$ was read a number of times. The value of this was invariable (0.017) throughout the observation period, — an increased safeguard as regards uniform registration and against irrelevant extinction changes.

Activity determinations on uricase suspensions

The velocity of the fall of the extinction which is caused by the addition of a certain amount of uricase to the mentioned glycine-buffered uric acid solution furnishes a measure of the uricase activity. The period $t_{\frac{1}{2}}$ in which half of the decrease is reached, may be read on coordinates or determined by interpolation. The initial velocity V_i is then defined by the equation

$$V_i = -\Delta E_{293\,m\mu}/2t_{\frac{1}{2}} .$$

A suspension of uricase is said to have the *standard activity* of unity if V_i is 0.100 per minute when 10 μl of the enzyme suspension is added to 3 ml of a mixture of uric acid and glycine buffer of pH 9.4. For clini-
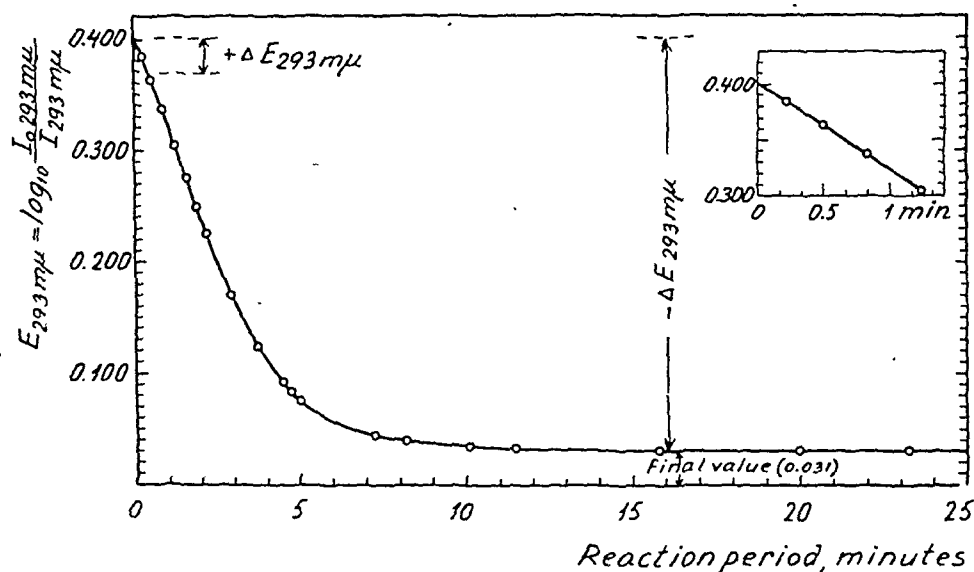
Fig. 2. The extinction change at 293 mμ produced by uricase added at zero time to a solution of uric acid containing 4.93 μg per ml. pH 9.4. Abscissae: reaction period, minutes. Ordinates: extinction at 293 mμ. Before the addition of uricase $E_{293\,m\mu}$ is 0.370. In the upper corner to the right: the initial part of the curve (the unit of abscisse is longer).

cal uric acid analyses 10 μl samples of suspensions with a standard activity of 0.2 —1.0 are preferable.

DETERMINATIONS OF URIC ACID IN BIOLOGICAL MATERIALS

The principle of the optic-enzymatic determination of uric acid in dilutions of blood plasma, serum, spinal fluid, sweat, tears etc. does not differ from the above measurement demonstrated in pure systems of uric acid, buffer and enzyme.

Urine

Each of 3 volumetric flasks contains 50 μl urine as well as 0.50 and 100 μl, respectively of a 0.02 % solution of uric acid added before the volume is made up to 10 ml with M/15 glycine buffer of pH 9.4. All of the flasks

contain, therefore, uric acid at a concentration which is 1/200 of that of the urine, the last two with a surplus of 1 and 2 μg added uric acid per ml of the diluted urine. With glycine buffer in the reference cell the readings were as entered in Table I, and the fall of the extinction at 293 mμ, upon the addition of uricase, is illustrated in Fig.3. —$\Delta E_{293\,m\mu}$ is 0.221, 0.366 and 0.442, respectively. If the first figure, representing the uric acid of the urine, is subtracted from each of the following two, the difference will be 0.075 and 0.151 representing the added uric acid, the concentration of which is 1 and 2 μg per ml, respectively. This is in close agreement with the above stated value of $K_{293\,m\mu}$, (0.0745).

In this experiment fresh urine was used. Four hours later the urine was very cloudy

Table I.

Determination and recovery of uric acid in urine. Extinction readings (293 mμ), at different times.

	Before enzyme	Extrapo-lation	$+ E_{293m\mu}$	Final value	$- E_{293m\mu}$
a. Diluted urine without added uric acid	0.336	0.353	0.017	0.062	0.291
b. with 1 µg/ml of added uric acid	0.417	0.431	0.014	0.065	0.366
c. with 2 µg/ml of added uric acid	0.490	0.508	0.018	0.066	0.442
d. Diluted urine 4 hours later	0.360	0.376	0.016	0.085	0.291
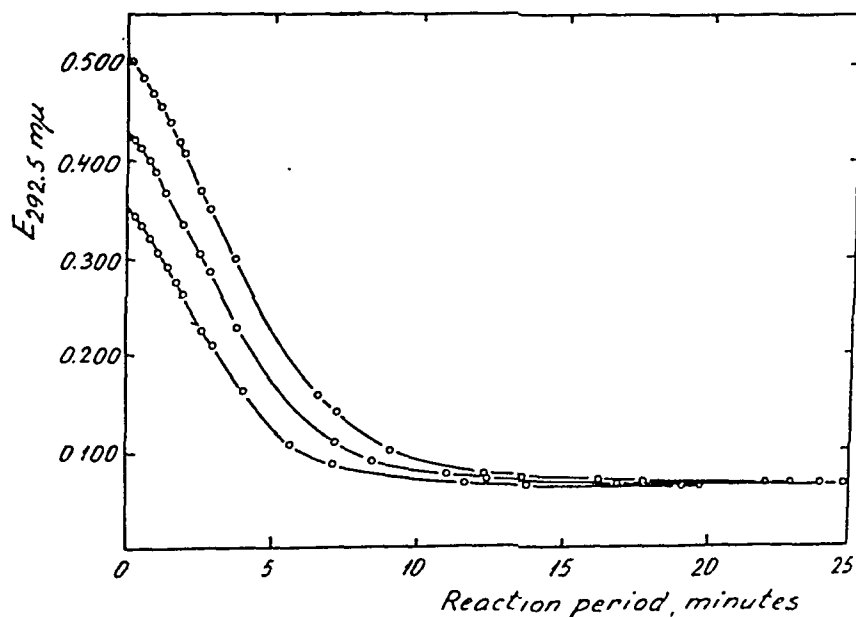
Fig. 3. Determination and recovery of uric acid in urine, diluted 1/200 with glycine buffer of pH 9.4. Reference cell contains water. Abscissae: reaction period, minutes. Ordinates: read extinction at 293 mμ.

Table II.

Determinations and recovery of uric acid in plasma.

	Before enzyme	Extrapo-lation	$+ E_{293m\mu}$	Final value	$- E_{293m\mu}$
a. Diluted plasma without added uric acid	0.224	0.250	0.026	0.160	0.090
b. with 0.975 μg/ml of added uric acid	0.293	0.321	0.028	0.160	0.161
c. with 1.95 μg/ml of added uric acid	0.362	0.393	0.031	0.161	0.232
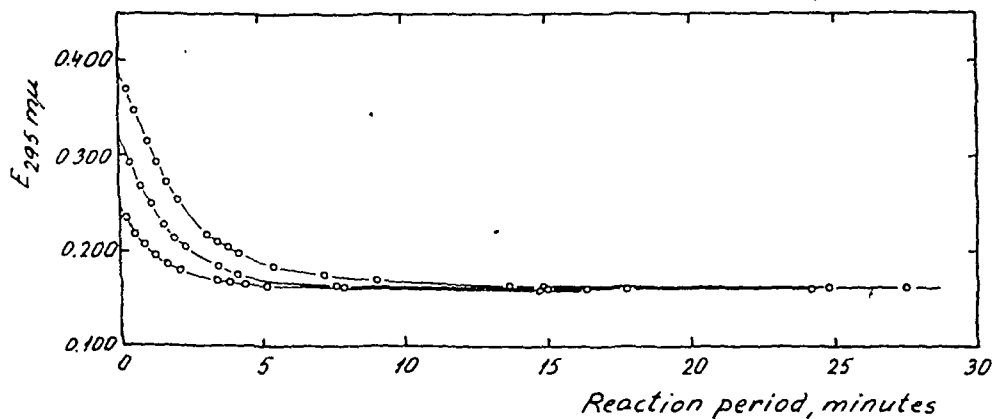
Fig. 4. Determination and recovery of uric acid in plasma, diluted 1/50 with glycine buffer of pH 9.4. Reference cell: about 10 μg uric acid per ml of the same buffer. Abscissae: reaction period. minutes. Ordinates: read extinction at 293 mμ.

and turbid, a new dilution 1/200 gave just the same value of $- \Delta E_{293 m\mu}$ as before, namely 0.291. The diluted urine therefore contained $0.291/0.0745 = 3.91$ μg uric acid per ml, and the urine 0.78 mg per liter.

Plasma

Each of 3 volumetric flasks contains 200 μl of heparin-stabilised plasma as well as 0, 9.75 and twice 9.75 μl, respectively, of the stock solution of uric acid, added before the volume is made up to 10 ml with the glycine buffer

of M/15. All of the flasks contain, therefore, uric acid at a concentration which is 1/50 of that of the plasma, the last two with a surplus of 0.975 and 1.95 μg added uric acid per ml of the diluted plasma.

With water or buffer in the reference cell the extinctions of the three solutions are too high to be accurately read. In Table II and Fig. 4 the readings are made against a dilution of the uric acid standard, (the reference cell contained about 10 μg uric acid per ml).

— $\Delta E_{295\,m\mu}$ is 0.090, 0.161 and 232, re-, spectively. If the first figure, representing the uric acid of the plasma, is subtracted from each of the following two, the difference will be 0.071 and 0.142. From this $K_{295\,m\mu}$ is calculated to be 0.729, which is the same value found in protein-free systems Præ-torius 1949).

The diluted plasma contained 0.090/0.729 = 1.23 μg uric acid per ml and the undiluted plasma, therefore, 6.15 mg uric acid per 100 ml. In this experiments the readings were made at 295 $m\mu$ instead of the usual 293 $m\mu$. Several other experiments at 293 $m\mu$ have shown, that also $K_{293\,m\mu}$ has the same value in systems with plasma or serum as in the case of protein-free systems. (0.0745). Although 295 $m\mu$ offers the advantage of a somewhat lower absorption by the plasma proteins, the wave-length 293 $m\mu$ should be recommended for routine determinations of uric acid in the clinical laboratory.

Spinal fluid

In the case of spinal fluid, which be diluted 1/3. the extinction may be read against the buffer unless the protein content is very high. If quite fresh spinal fluid is examined, however, the extinction at 293 $m\mu$, before the addition of uricase, is not quite constant. The spectral change covers a region in the ultraviolet from 240 to 300 $m\mu$ and is due to an unknown reaction which proceeds much faster when borate buffer is used instead of glycine buffer. In the last case the change takes several hours while in borate buffer the lowering of the extinction, which is most pronounced at 260—265 $m\mu$. has completely ceased in less than two hours. Determina-

tions may then be carried out just as described for urine. The normal range of concentration is 0.15—0.45 mg uric acid per 100 ml of spinal fluid. With non-enzymatic methods the content of uric acid in the spinal fluid is found 5—10 times as high (Cockrill 1931).

DIRECTIONS IN DETAIL.

The activity of the enzyme must be adjusted by dilution of a stock suspension of uricase with M/15 glycine buffer of pH 9.4. The velocity of the decrease of the extinction at 293 $m\mu$, corresponding to the breakdown of uric acid, must not be so high that it is practically impossible to ensure an accurate extrapolation to zero time. On the other hand the conversion of uric acid to compounds without absorption must be finished in less than two hours. (See "Activity determinations".)

Dilution of the material to be examined is most accurately done by means of Carlsberg constriction pipettes and volumetric flasks. but can of course be carried out in the quartz cells, the contents of which in general should be at least 2.5 ml. A micromethod, however, which only requires 20 μl of plasma diluted to 1 ml, has been elaborated (not published). In this method a diaphragm with a circular opening reduces the diameter of the light beam to 5 mm (compare Lowry & Bessey 1946). and the (standard) quartz cell is elevated by a wooden block of about 10 mm hight.

If 134 μl of the material is diluted to ten ml the uric acid content in mg per cent is obtained by muliplication of $-\Delta E_{293\,m\mu}$ by 100. In the case of plasma or serum it

is preferable, however, to use pipettes delivering the double amount (exactly 268½ μl), in normally concentrated urine 67 μl.*

The contents of the reference cell may be water or buffer if the concentration of protein of the sample is low. In the case of plasma or serum, however, the extinction difference between the measuring cell containing the diluted sample and the reference cell must be lowered in order to get readings on the transmission scale in the region which offers the highest reading accuracy, i. e. below 0.400. 4 μl of Folin's Stock solution of uric acid added to 2.5 ml buffer in the reference cell will reduce the reading about 0.100. If the plasma is diluted 1/40 20—25 μl of the stock solution will be an adequate amount to add to the buffer in the reference cell.

The enzymatic reaction is started by the addition of about 10 μl of uricase suspension and stirring by means of a plastic rod, the end of which is somewhat flattened and broadened. The enzyme addition and the stirring should take place simultaneously when the second-hand of a watch passes zero and 4—5 extinction readings are made in rapid succession to ensure an accurate extrapolation to zero time.

The final extinction value must be controlled at intervals of ten minutes or more, especially when the standard activity of the enzyme is low (compare the curves in Figs. 2—4).

The slit-width is nearly proportional to the width of the spectral band at a given wave-length. If the wave-length scale is in the position 292.5 mμ and the slit-width amounts to 1.2 mm, a spectral band of 290—295 mμ will pass through the cells. From a comparison with the spectrum of uric acid (Fig. 1) it is obvious that the value of K must be redetermined if the slit-width exceeds 1.2 mm.

Working capacity of the method. In serial determination two persons (one reads another writes) may determine uric acid in 10 samples per hour if a sufficient number of quartz cells are at disposal (6—10). Even under the conditions of rapid working the deviation from mean did not exceed 2.5 % in an experiment in which the reproducibility of the method was to be tested.

SUMMARY

A specific, accurate and rapid method for the determination of uric acid in blood, urine and spinal fluid is presented. It is based on enzymatic ultraviolet differential spectrophotometry. Uric acid produces a very strong absorption of ultraviolet light, especially between the wave-lengths 290 and 295 mμ. This absorption disappears completely upon the addition of a small amount of purified uricase. The extinction difference before and after the action of the enzyme is a direct measure of the amount of uric acid of the sample. Deproteinization is not necessary and should be omitted. The method permits about 10 determinations per hour with a reproducibility of ± 3 %.

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EXPERIMENTAL MACROCYTIC ANÆMIA IN THE RAT

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The experimental study of the pathogenesis of pernicious anæmia is extremely difficult, since it seems almost impossible to induce this disease in laboratory animals. For this reason it has also been very difficult to elaborate a convenient test method for the therapeutic potency of liver extracts other than their clinical trial.

During the last twenty years many tests have been suggested fo rthe assay of liver extracts; Eisler et al. (1936—37) injected hydroxylamine into rabbits and found that the anæmia thus produced would respond to liver extracts. Jacobsen, Milwertz and Plum (1939) found in the meantime that this method was unsuitable for the biological assay of liver preparations therapeutically active in human pernicious anæmia. Later King (1947) has reviewed the tests made on an experimental basis, and points out that none has been proved of value.

The observations of Castle (1929) suggested that pernicious anæmia might be produced experimentally by resection of the stomach. In man, total gastrectomy is often followed by severe anæmia. Nørgård (1937), Leroux & Vermés (1939) describd 29 cases of pernicious anæmia after gastrectomy, but in most cases this anæmia following gastrectomy was microcytic. Many experiments have been carried out on animals, (Jung et al., 1932—33, Ivy, 1940 Jones, 1940 Jacobsen & Plum, 1948), but also here the anæmia produced is very seldom of the pernicious type and one might say that a blood picture corresponding to that of human pernicious anæmia is never seen in animals.

Many experiments have been carried out in order to investigate the function of the intestine and its relation to blood formation, and it has been found that resection of the small intestine in man in some cases is followed by some sort of pernicious anæmia (Ryti, 1927, Glatzel, 1929, Becker, 1930, Strandell, 1931, Fleischhacker & Klima, 1936, Holmgren, 1944), in animals many experiments have also been carried out, but mostly in vain (Petri & Jensenius, 1941, Jensenius, 1945, the latter giving a review of the literature).

Examinations of the functions of the large intestine and its possible relation to blood formation have also been carried out. Ever since Faber (1897) made the first observations on diseases of the large intestine and macrocytic anæmia, many similar cases have been reported. Barker & Hummel (1939) collected 51 cases of macrocytic anæmia in association with intestinal stricture or anastomosis, and in the work of Jen-

senius (1945) other cases have been reported. In all these last cases it has been impossible to separate this type of anæmia from the usual pernicious anæmia because the bone-marrow was usually megaloblastic. There are, however, certain differences between pernicious anæmia and the anæmia caused by intestinal stricture or anastomosis — there is no achlorhydria or leucopenia, and neurological symptoms are absent or at least very rare. Liver extract therapy was useful.

In 1948 Watson et al. described at method by means of which it was possible to produce a macrocytic anæmia in rats, an anæmia which responds to treatment with liver. The principle was that they made a blind intestinal loop in rats. The pathogenesis of this anæmia is not yet known, but it seems to depend upon dilatation of this blind loop, with succeeding accumulation of intestinal content. When the paper of Watson et al. was published, I was carrying out some experiments in order to elucidate the function of the coecum in blood formation and these experiments were continued. The present paper deals with blood formation in normal rats after resection of the coecum, and the effect of liver extract and other therapeutics.

METHODS

The rats used in all the following experiments were albinos of the Wistar strain and only male animals were used. The age of the animals was 4 months. Each animal was kept in its own box, and blood examinations were performed twice at an interval of at least 14 days. After the last examination the operation was made. After the operation the animals were examined at first once every fortnight and after 56 days the examination was made only once every 28th day.

The animals were kept on a normal diet without cabbage and lettuce, sometimes they got some car-

rots. The controls kept on the same diet for the same period did not develop anæmia and showed normal growth.

The resection of the coecum was made after anaesthetizing the animal with "Cytodan-natrium, Leo". The animal was fastened on its back on the operating table. An incision of 3 to 4 cm was made in the mid-line of the lower abdomen, the coecum thus becoming visible in the middle of the operation field. With great care the end of the coecum was found and all the vessels supplying the coecum were ligated and cut from the end to the ilio-coecal orifice. Then the colon and ileum were anastomosed by end to end anastomosis. The abdominal cavity was then closed, first the peritoneum and the muscles and then skin. Usually the animals "slept" 3 to 5 hours afterwards.

RESULTS

Nearly all the animals display anæmia after the operation. In most cases this anæmia is of the microcytic type, which reacts to iron, but about 40 per cent show a macrocytic anæmia with anisocytosis and poikilocytosis. The mortality is high during the first two weeks after the operation, and afterwards the animals either remain well or they progressively develop a severe anæmia, and die, if no treatment is administered. The hematological findings are given in Table I. The normal hemoglobin level in rats from our strain is 102 per cent. (100 per cent = 13.5 g per 100 ml) and an anæmia cannot be diagnosed unless the hemoglobin has fallen below 80 per cent. Very often a slight initial anæmia is found in animals operated on as described, and here the colour-index may be increased, i. e. a hyperchromic anæmia.

The red blood cell count which is normally a little less than 7,000,000 pr. cmm, often (35 per cent) shows a fall at the same rate as the hemoglobin with the result that the

Table I. *Variations in the number of red blood cells, hemoglobin and the diameter of the red blood cells in rats after resection of the coecum. The standard deviation for hemoglobin is ± 10, for erythrocytes ± 0.41 and for the diameter ± 0.09.*

Days	Number of animals	Hemoglobin 100 % = 13.5 g per 100 ml	Erythrocytes in mill per c.mm.	Diameter of the red blood cells 1.0 = 1.08 μ
0	100	102 . (93 — 123)	6.71 (5.88 — 7.34)	5 88 (5.70 — 6.05)
14	64	97 (82 — 117)	6.82 (5.09 — 7 99)	5.92 (5.59 — 6.23)
28	57	94 (76 — 107)	6.90 (4.72 — 7.88)	6.07 (5.29 — 6.52)
42	57	84 (60 — 102)	6 70 (4.09 — 7.29)	6.20 (5.46 — 6.47)
56	46	82 (52 -- 108)	6 42 (4.27 — 7.37)	6.32 (5.44 — 6.52)
84	33	80 (50 — 107)	6.27 (3.19 — 7.09)	6.19 (5.20 — 6.59)
112	31	76 (43 — 106)	6.13 (2.27 — 6 94)	6.12 (5.39 — 6.80)
140	31	79 (40 — 110)	5.83 (2.42 — 6.73)	6.32 . (5.20 — 6.92)
168	29	83 (42 — 100)	5.71 (2 38 — 6.69)	6.22 (5.14 — 6.87)
196	27	75 (38 — 108)	5.64 (2.18 — 6.57)	5.37 (5.27 — 6.27)
Controls 196	25	99 (90 — 129)	6.68 (5.93 — 7.23)	5.86 (5.70 — 6.02)

colour index (Hemoglobin percentage: Number of erythrocytes in mill. × 15.0) does not change. In about 25 per cent of the animals used for these investigations the hemoglobin decreased more rapidly than the red blood cells and here a decreased colour-index is found — and only iron therapy was useful. In 40 per cent the decrease of the red blood cells is very rapid, and an increase in colour-index was found. The normal mean red blood cell diameter was found to be 6.35 μ. The presence of macrocytosis has been shown by an increase of the mean cell diameter and by an extension of the Price-Jones curve beyond the upper limits of the

normal, and in the anæmic rats where an increase in colour-index was found there was also an increase in the mean cell diameter from 6.35 μ to 7.47 μ (Table I and Fig. 1). Anisocytosis was a striking feature of the anæmia, but poikilocytosis was very seldom observed. In the animals where a decrease in the colour-index was found, the mean cell diameter was also decreased (Fig. 2).

Most of the anæmic animals showed an irregular reticulocytosis manifesting itself in the stained smears by increased polychromasia and also in reticulocyte counts in moist chamber. Normally only a few nucleated red cells were present in the peripheral
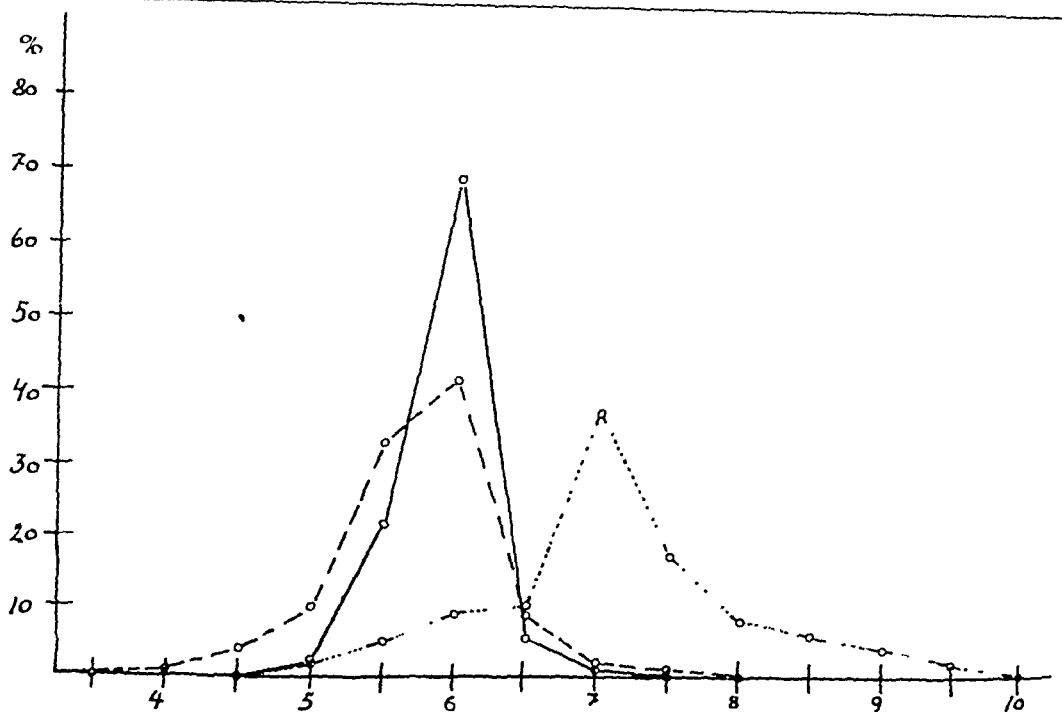
Fig. 1. The Price-Jones curves in rats. ——— normal, . macrocytic anæmia, — — — microcytic anæmia. Abscissa Diameter (in *n* multiplied with 1 08) Ordinate Number of cells in per cent.
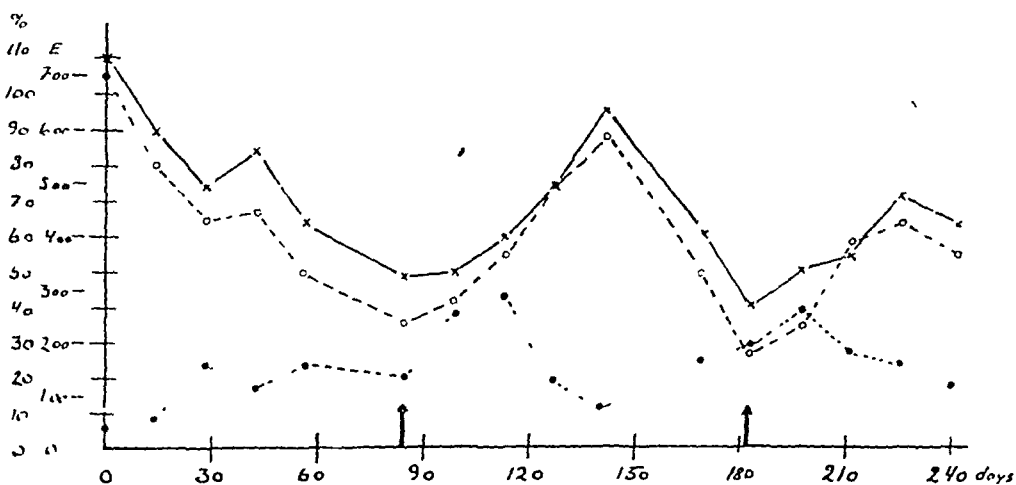
Fig. 2 Variations in the amount of red blood cells hemoglobin and reticulocytes in a rat after resection of the coecum and the response to liver extract Abscissa Time in days.

Ordinate ×——× Hemoglobin in percentage.
o – – –o Erythrocytes in mill per cmm
— – – Reticulocytes per 1000 erythrocytes
↑ Injection of liver extract.

Table II. *Normal red blood pictures given by different authors.*

	Watson et al. (1948)	Andreasen (1943)	Plum (present paper)
Hemoglobin	100% = 13.8 g	115% 100 — 120	102% (100% = 13.5 g) 93 — 123
Erythrocytes in mill. per c.mm ...	8 69	7.6 6.3 — 8.9	6.71 5.88 — 7.34
Colour-index	0.77*	1.01 0.9 — 1.19	1.01 0 93 — 1.11

* Calculated by the present author.

blood smears, but after operation an increase was observed.

The leukocytes were present in normal numbers, or a slight increase was found, especially during the first two weeks after the operation. Most striking were the neutrophilic granulocytes. The cells showed a more differentiated picture; in the operated animals it was rare after 3 to 4 weeks to find the granulocytes with "ring-shaped" nucleus normally found in rat blood.

The blood picture found in the rats used for the experiments in this paper is very much like that described by Watson et al. (1948), but there are differences which ought to be mentioned. The blood picture in the normal rats differs, and in Table II I have compared the normal values.

It will be seen that there is a great difference in the colour-index, and when one calculates the colour-index in the material given by Watson et al. only four of the animals show a colour index outside the limit of the normal value ± 10 per cent and only two are within the limits of the values given by Andreasen and Plum, all other values being below the lower limit. It is difficult to explain these findings and especially to say anything about the consequence, except that the results are not directly comparable.

The bone marrow

Bone-marrow smears were made from normal and anæmic animals obtained by puncturing the tibia just below the knee-joint. The normal bone-marrow showed a picture like that reported by Töppner (1942), Vogel (1927), and Cameron & Watson (1948). In smears from normal rats the predominant cells of the erythroblast series are small polychromatic normoblasts with a dense nucleus. In the operated animals, this distribution was unaltered if the anæmia which developed was normocytic or microcytic, but in rats with macrocytic anæmia, there was often an increased number of pro-erythroblasts and basophilic erythroblasts, a small number of cells corresponding exactly to the megaloblasts seen in human pernicious anæmia. In some animals the myeloid series showed the same picture as in human pernicious anæmia, but this picture was very rare and was only found in animals with a severe anæmia — these animals died shortly after the examination.

Effect of therapy

The main object of the experiment described above was to try to get a convenient method for the standardization of liver-extracts. The experiments carried out show that it is possible to produce a macrocytic
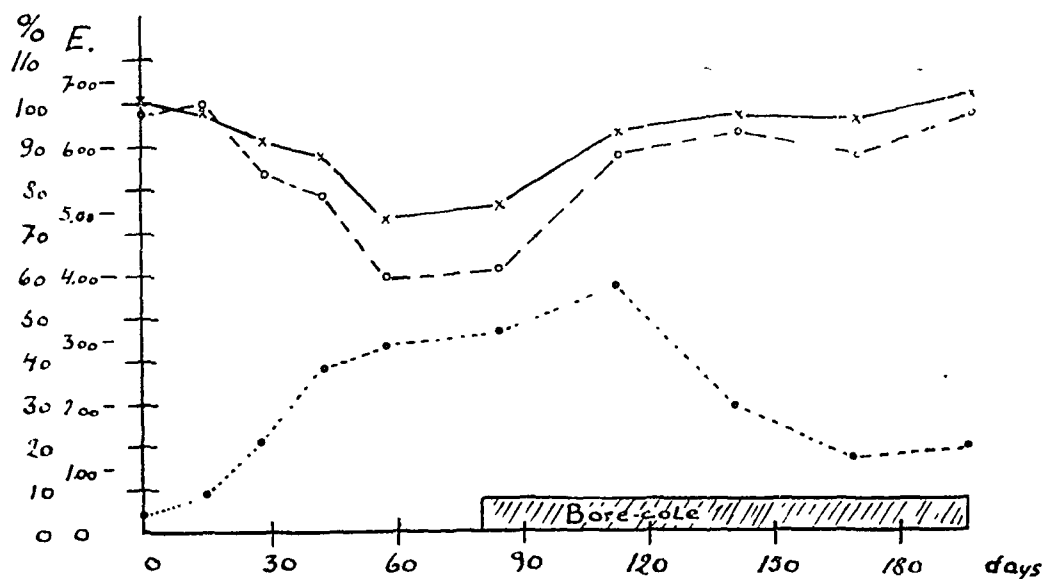
Fig. 3. Variations in the amount of red blood cells, hemoglobin and reticulocytes in a rat after resection of the coecum and the response to feeding with borecole. Abscissa: Time in days.
Ordinate: ×———× Hemoglobin per centage.
o————o Erythrocytes in mill. per cmm.
————. Reticulocytes per 1000 erythrocytes.

anæmia in rats, but it has also been shown that the occurrence of macrocytic anæmia is not a regular phenomenon, only 40 per cent of the examined rats develop a macrocytic anæmia. If the rat is to be used as a test object for the antipernicious principle, it is found, as pointed out by Watson (1948), that the percentage must decrease very slowly, if the decrease is too rapid the animal will die quickly even when therapy has begun. The best results are obtained when the hemoglobin is about 60 per cent, if the anæmia is more severe nutrition and resistance to infection are impaired.

Commercial preparations such as liver concentrate "Lederle" 15 U. P. and Hepsol fortior "MCO" were used to study the effect of liver extracts on macrocytic anæmia in rats; the extracts which were used had previ-

ously been tested clinically and found to be active. Usually 0.2 ml was given to each animal subcutaneously and each preparation was tried on at least 20 animals. The response to liver extracts varied considerably in degree, a typical response is given in Fig. 2.

In 14 out of 20 animals, Liver-concentrate "Lederle" (15 U. P.) caused a reticulocyte crisis and an increase in hemoglobin and later in the number of red blood cells. Of the 6 animals which did not respond, 3 had extensive septic infections, one had a stricture in the anastomosis and the autopsy of two did not reveal anything pathological. Liver extract Hepsol Fortior "MCO" showed that 10 of the animals responded with a reticulocyte crisis and increase of hemoglobin and erythrocytes Four of the

last 15 animals which did not respond had septic infections, 3 had a stricture in the anastomosis, and the autopsy of 8 did not reveal anything pathological.

If 0.3 ml was given instead of 0.2 ml Hepsol Fortior "MCO", the liver extract caused a reticulocyte crisis in 15 of the animals and increase in hemoglobin and red blood cells. Two of the 10 animals which did not respond had extensive septic infections, 2 had a stricture in the anastomosis and the autopsy of 6 did not reveal anything pathological.

When the liver extract was diluted 1 : 10, only 4 out of 20 animals responded in the same way as when the undiluted liver extract was used; when undiluted liver extract was given, 8 of the remaining 16 animals showed a weak secondary reticulocyte crisis.

In all these experiments the animals were kept on a diet without cabbage and lettuce, and it was incidentally found (Fig. 3) that when the diet was supplemented with borecole the usual macrocytic anæmia did not develop and if the borecole was given some weeks after the operation the animal responded and the anæmia was cured.

DISCUSSION

In a series of experiments investigating the rôle of different parts of the alimentary tract in blood formation, the function of the coecum was investigated, and it was found that in about 40 per cent of the animals (rats) a resection of the coecum was followed by a macrocytic anæmia. The anæmia observed in the rats resembled in most of its features the anæmia described in human cases of intestinal stricture and anastomosis. It is difficult to say anything about the anæmia produced in the rats and its relation to Addisonian pernicious anæmia.

Of great interest is the therapeutical effect of liver extracts and the amount of liver extract required for response. The doses used in the reported cases are smaller than those given by Watson et al. and this seems to show that there are differences between the macrocytic anæmia produced by Watson's blind intestinal loop and the anæmia produced by resection of the coecum. The latter seems to be somewhat more susceptible to liver extract.

Experiments carried out on rabbits seem, in a few cases, to show the same results found in rats, but here the great problem is to keep the animals on a diet without any cabbage, lettuce etc.

The manner in which liver extracts produce their effect is unknown, and we do not know anything exact about the amount of liver extract necessary for producing a remission in relation to body weight and to the life span of the red blood cells, and it seems that Watson et al. are right when they state: "Perhaps there is a relation between the life span of the red blood cells and the need for hæmopoietic principle." Perhaps there are greater differences between experimentally developed macrocytic anæmia and the pernicious anæmia found in man, and this is the reason why greater amounts of liver extracts are needed; the mechanism of the function of liver extracts may be quite different — there is for example no achylia.

SUMMARY

The resection of the coecum in rats causes a macrocytic anæmia in about 40 per cent of the animals operated. The anæmia responds to treatment with large doses of liver extract.

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APPARATUS AND METHODS FOR THE ESTIMATION OF PULMONARY FUNCTION

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APPARATUS

The apparatus to be described in the present paper has been used for some years in this department, in various attempts toward the evaluation of pulmonary function. Originally conceived by Carl Sonne (sketched in a paper (Sonne 1940) as part of an apparatus constructed for obtaining fractionated samples of alveolar air) it was applied later to investigations on the changes in the resting respiratory level (Sonne and Georg 1945), and has now with some modifications proved a useful means in the testing of pulmonary function.

The spirometers in general use (i. e. the oxygen containing, closed-circuit types) have two disadvantages in investigations of this type: that the experiments are bound to be carried out during oxygen-breathing, and that the working experiments have to be interrupted after one or two minutes respiration, owing to the great increase in oxygen consumption. The merits of the present apparatus are that the respiration can be carried out in atmospheric air (or oxygen or any air-mixture if wanted), and that the spirogram can be recorded continuously for a considerable time. This is achieved simply by an absolute separation of inspiratory and expiratory air.[1]
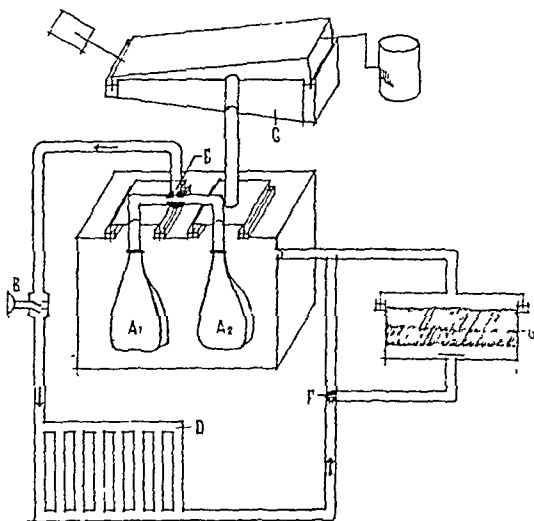
Fig. 1. Schematic illustration of the apparatus. (See text for detailed description).

In Fig. 1 the apparatus is represented schematically. It consists of a wooden box (measuring 80 × 80 × 120 cm) in which two Douglas-bags (A_1—A_2) are suspended (each bag measuring 150 litres). The Douglas-bags are introduced through the roof of the box, and the airtight condition of the suspending system secured by water-traps.

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[1] *Addition to proof* The same principle has been used in an apparatus recently published by Donald and Christie (Clin. Science 8, 21, 1949).

The patient breathes through a mouth-piece and rubber valves (B), drawing the inspiratory air from the bags, and expiring into the box (outside the Douglas-bags). During the experiment the air is thus shifted from the bags to the interior of the box, without any intermingling between inspiratory and expiratory air. The respiratory tracing is obtained by means of a Krogh-spirometer (C), intimately connected with the interior of the box through a wide rubber tube (6 cm internal diameter). On the tracing — recorded as usual by an ink writer on a rotating drum — the respiratory curve takes an almost horizontal, slightly downward slope. (This is due to the respiratory quotient being a little below unity). The respiration can be recorded without interruption until the two Douglas-bags are emptied. i. e. until the entire ventilation amounts to 300 litres. This suffices for about 30 minutes resting respiration or about 10 minutes of an ordinary exercise test.

On the expiratory tube is inserted a hollow copper-grate (D) (containing 30 pipes, 2 cm wide, 1 meter high) which guarantees the constant temperature of the apparatus. In earlier experiments, before this grate was used, it was found that the expiratory air was slightly heated, causing an upward slope of the respiratory tracing in the working experiments. Now the temperatures of the inspiratory and expiratory air remain identical even with ventilations in the magnitude of 60 to 80 litres per minute.

The apparatus is furnished with two three-way cocks. The first one (E on the figure) is placed at the connection of the inspiratory tube with the Douglas-bags. It allows the exclusion of one or the other of the bags, thus enabling the examiner to introduce in-

spiratory air of altered composition. Carried out during an expiration, the change is imperceptible to the subject. The other cock (F on the figure) introduces a soda-lime canister (G) for the absorption of carbon dioxide, to be used in determinations of the metabolic rate.

In its present form the apparatus also records continuously the respiratory minute-volume of the subject. This is achieved by means of a device, originally described by Tybjærg Hansen (1945) as a "ventilation adder", later modified for automatic recording (Sonne and Georg 1949).

FROM OXYGEN CYLINDER ————
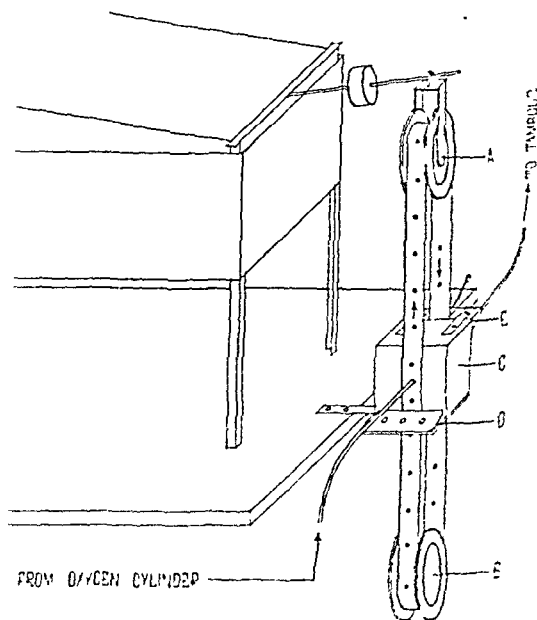
Fig. 2. Schematic illustration of the ventilation measurer. (See text for detailed description).

Fig. 2 presents schematically this part of the apparatus. On the arm of the counterpoise of the spirometer is suspended a pulley (A), carrying a paper ribbon 2 cm wide (The diameter of the pulley is 3.5 cm.) The ends of the ribbon are connected, forming a noose in which a second similar pulley (B)

rests, thus constantly keeping the ribbon taut. The paper passes closely along a wooden block (C), against which two razor-blades (D and E) regulate the movements of the ribbon, enabling it to move in one direction only. Thus every up and down movement of the spirometer bell during respiration is transformed into a displacement of the paper ribbon in one direction, the displacement being proportionate to the ventilation of the subject. Now the paper ribbon is furnished with holes placed equidistantly, the distance between two holes corresponding to a certain ventilation, e. g. 5 or 10 litres. (This distance may either be calculated or determined empirically). From a cylinder containing oxygen (or atmospheric air) a slight stream of air is directed through a piece of rubber tubing (1—2 mm diameter) toward the middle of the paper ribbon. Each time a hole passes this stream of air, the air continues through a small metal-tubing (passing obliquely through the wooden block) into a tambour, which marks on the kymograph drum.

A spirogram as recorded by the apparatus is presented in Fig. 3. Below on the figure
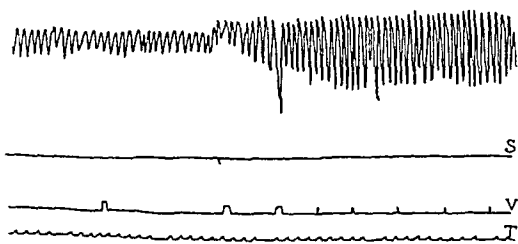
Fig. 3. Part of a typical spirogram as recorded by the apparatus. S: signal-line, indicating the beginning of work. V: curve of the ventilation measurer, the distance between each jag representing 10 litres. T: time-marking, tenths of a minute, every tenth stroke omitted.

the time-marking is found (being in tenths of a minute, every tenth stroke omitted), and above that the curve of the ventilation-measurer, the distance between each jag equalling 10 litres. In the respiratory tracing inspiration is recorded downstroke, expiration upstroke. The signal line indicates the beginning of work.

METHODS

The principal task of the lungs is to arterialize the blood, i. e. to saturate the venous blood with oxygen and to remove from it a sufficient amount of carbon dioxide. As carbon dioxide passes through the alveolar membrane about 20 to 30 times as fast as does oxygen (Krogh 1919) it is the saturation with oxygen which is primarily found to be faulty in cases of pulmonary insufficiency. Thus pulmonary insufficiency can be said to exist when the oxygen saturation in the pulmonary veins is below normal, either at rest or — in less pronounced cases — during exertion.

For ordinary purposes the oxygen saturation of the blood in the pulmonary veins is identical to that of the arterial blood, — the obvious exceptions being the cases of venous-arterial shunt encountered in congenital cardiac disease or cavernous haemangioma of the lung. Therefore the determination of the arterial oxygen saturation is of principal interest in the evaluation of pulmonary function.

The connexion between respiration and circulation is so intimate, however, that a deterioration of pulmonary function sooner or later leads to an increased stress on the circulation (i. e. on the right heart). An insufficient circulation of blood through the

lungs may thus conceal the pulmonary insufficiency in reducing the arterial hypoxemia or making it disappear. Thus it follows that the only certain deduction to be drawn from a decrease in arterial oxygen saturation during work (the aforementioned instances of venous-arterial shunt being excluded) is that pulmonary insufficiency is predominant in the insufficiency existing. It may be a case of isolated pulmonary insufficiency, or it may be a case of combined cardio-pulmonary insufficiency (in which the pulmonary factor is the prevailing). Other tests are needed to determine this.

In the estimations of pulmonary function as carried out in this department the following procedure has been adopted:

First the subject is examined in the morning in bed, under conditions identical to those required for the determination of the basal metabolic rate. The respiration is recorded for about 12 minutes, the subject respiring for the first 5—6 minutes in atmospheric air, and later in oxygen. From the tracing the respiratory frequency, minute-ventilation and oxygen consumption are calculated, for atmospheric air as well as for oxygen. Furthermore an estimation of the arterial oxygen saturation is carried out, by means of an oximeter of automatic adjusting type (Millikan 1942). The oximeter is set at 100 % during the oxygen breathing, and the values recorded at two-minute intervals after the interruption of the experiment, until the oxygen saturation remains constant. (The assumption that the oxygen saturation of the arterial blood is 100 % when the subject respires in pure oxygen does of course not always hold true. In the occurrence of venous-arterial shunts this is not so,

and in more severe cases of pulmonary disease with perfusion of non-aërated parts of the lungs this may not be so either. The physically dissolved oxygen, taken up during respiration in pure oxygen, is however sufficient to cope with smaller shunts of this sort. Under such conditions an admixture of about 25 % venous blood is necessary before any decrease in the chemically bound oxygen is likely to occur. (Cf. Berggren (1942) for a discussion of this.))

At the next stage a determination of lung volume and its subdivisions is carried out, — a hydrogen-oxygen mixture being used for the estimation of residual air, and the maximum breathing capacity is determined. The author shares with others the appraisal of the ratio: residual air/total capacity as a most valuable measure in the evaluation of pulmonary function. The determination of the maximum breathing capacity, granting its theoretical value, has the disadvantage of depending entirely on the subject's will to coöperate. With this condition satisfied the test offers most useful information, however.

At third stage of the examination the subject performs graduated exercise on a bicycle ergometer (Krogh 1913), the respiration being recorded by the apparatus as usual, and the changes in arterial oxygen saturation followed by the oximeter. (While the accuracy of the absolute values recorded by the oximeter has been open to criticism (Wood and Geracy 1949) the instrument is a reliable and sensitive indicator of changes in oxygen saturation). The intensities of work performed are usually 400 and 800 kgm minute. The exercise has to be carried out for 3—4 minutes only, viz. the time

necessary to realize wheher the subject is able to obtain a steady state or not. With normal subjects the author has never recorded any decrease in arterial oxygen saturation during exercise of the intensities mentioned, but at more exhausting work even normal persons may exhibit a slight decrease in arterial oxygen saturation. The diminutions recorded in subjects with pulmonary disease are usually within 5—10 %, while in cases of congenital cardiac disease with venous-arterial shunt the decrease in arterial oxygen saturation during even a slight exertion is far greater, 20—30 % or more being the rule.

In general it is in pulmonary disease of not too long standing (e. g. young people with emphysema, fresh cases of silicosis etc.) that a decrease in arterial oxygen saturation during exertion is most pronounced. The more pronounced the chronic, fibrotic character of the disease, the more is the right heart affected and the pulmonary insufficiency complicated by developing cardiac insufficiency. Therefore the working test with the oximeter is only one of several tests of pulmonary function which must be applied to secure a reliable evaluation of a given case of pulmonary disease.

The tests of pulmonary function have found their principal use as means for the objective estimation of the patient's disability: in diseases in which economic compensation is applied for (e. g. silicosis), or in the study of the results of specific treatment (e. g. for sarcoidosis or pulmonary tuberculosis). In dyspnoic states of uncertain origin an evaluation of pulmonary function is of diagnostic interest.

The following case is cited as an example of a typical examination: The patient is a 25 year old woman, admitted for sarcoidosis of the lungs. Complaints: slight, not productive cough, moderate dyspnoea on exertion. X-ray: enlarged hilar shadows with mottling of lung fields on both sides.

Results of function test:

Vital capacity 2.15 litres (77 % of total capacity), residual air 0.65 litres (23 % of total capacity: 2.80 litres) (Predicted value for total capacity: 4.30 litres.)

Maximum breathing capacity: 36—36—40 litres per minute.

Ventilation at rest: 5.4—5.6 litres per minute, frequency 17—18.

Arterial oxygen saturation at rest: 95 %.

During exertion of 300 kilogrammetres per minute a decrease in arterial oxygen saturation of 4—5 % is observed, the ventilation being 18—19 litres per minute, frequency 24. A heavier exertion (600 kilogrammetres per minute) is interrupted by the patient after 1 minute.

Conclusion: Moderate decrease of functional ability with predominating pulmonary insufficiency, corresponding to a fibrotic pulmonary disease with low total capacity and rather poor ventilatory function. No emphysema.

In the opinion of the author the tests outlined here will in most cases enable the investigator to obtain a fairly satisfactory estimation of the degree of pulmonary insufficiency. A further discussion of the results is beyond the scope of the present paper.

SUMMARY

Description is given of an apparatus which for some years has been employed in the spirometric examinations carried out in this department. The apparatus records continuously the respiration and respiratory minute volume of the subject, who breathes inspiratory air of unchanging composition.

The composition of the inspiratory air may be chosen at will, and the subject may be switched imperceptibly from one sort of inspiratory air to another (e. g. from atmospheric air to oxygen, or vice versa).

The procedure adopted for the estimation of pulmonary function is outlined. In the evaluation of the results special stress is laid upon the values for the ratio: residual air; total capacity, for the maximum breathing capacity, and upon any decrease in arterial oxygen saturation during exertion (the oxygen saturation being recorded by a Millikan-oximeter).

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KETONE METABOLISM IN HEALTH AND IN HEPATIC DISEASE

I. KETONE METABOLISM IN NORMAL SUBJECTS

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In a previous paper (1947) the author presented a rather thorough physiological survey of the rôle of the ketone bodies in metabolism. Other reviews have been made by Peters & van slyke in "Quantitative Clinical Chemistry", Vol. I (1945) and by Duncan in "Diseases of Metabolism" (1948).

Despite numerous and extensive studies several points concerning the ketone bodies are still not clear and it is exceedingly difficult, in the light of present knowledge, to evaluate the rôle of the ketone bodies in intermediary metabolism. The present knowledge of the rôle of ketone bodies in metabolism may be summarized as follows.

Presumably the ketone bodies are *normal* intermediate products in the conversion of the fatty acids and of certain ketogenic amino acids, the chief production, however, being derived from the fatty acids. The ketone bodies are *formed* in the liver — probably here exclusively — but are *decomposed* in all tissues, particularly in muscles and kidneys. The ketonaemia occurring in a variety of conditions cannot be attributed to lowered combustion in the extrahepatic tissues, but is due to a transient increase of ketone production in the liver. The higher concentration of ketone bodies facilitates oxidation in the tissues. The inter-relationship between inadequate carbohydrate metabolism and ketonaemia — of which there is clinical proof — is hardly due to coupled processes in which an intermediary metabolite of carbohydrate (pyruvic acid) is supposed to act as hydrogen acceptor in the dehydrogenation of the ketone bodies, but merely to the fact that fat metabolism predominates.

In the modern sense of the word *antiketogenic substances are substances which througt their own combustion exercise an economizing effect on fat metabolism.* Therefore, they are not identical with glycogenetic substances .

Whereas it is almost certain that ketone bodies are *normal* substances it is difficult to decide whether they are *obligatory* conversion products of the fatty acids. So far no direct proof of the method of ketone formation has been set forth, but it is worth mentioning that recent investigations in which marked carbon atoms were used, strongly indicate the formation — at the intermediary stage of ketone production — of *compounds containing two carbon atoms only.* This implies that the theory of β-oxidation — although in a different manner than originally assumed — may be correct. (Weinhouse, Medes & Floyd 1945.)

There seems to be sufficient proof that the ketone bodies are useful substances, as the amount of ketone bodies formed in the liver is in excess of the amount excreted in the urine. Further, the peripheral tissues

(muscles) are able to oxidize ketone bodies. It has now been possible to prove conclusively that the ketone bodies may undergo active reabsorption in the kidneys, but it may be assumed that the renal threshold for ketone bodies is very low as compared with normal concentrations in the blood.

The capacity of normal men and animals to form ketone bodies has been the subject of numerous investigations. The most extreme degrees of ketone formation in animals were encountered in experiments with pancreatectomized animals, i. e. in pathological states. Yet, it cannot be said that there is any fundamental difference between the formation of ketone bodies in these animals and in normal animals maintained on a high fat diet.

The investigator desirous of studying ketone metabolism in normal subjects may employ either inanition or a high fat diet.

PERSONAL INVESTIGATIONS

Ketone metabolism in normal subjects was studied 1) by producing inanition ketonaemia, 2) by administering a ketogenic diet. As mentioned previously in this report there is no reason to differentiate, as did some earlier workers, between endogenous and exogenous ketogenesis, as *there is no fundamental difference between the two forms of ketone formation.*

The subjects studied revealed no indication of hepatic disease, diabetes, myopathy, adipositas or extreme emaciation. They were patients who had been suffering from neurasthenia, gastritis, constipation, sciatica, angina, rheumatic fever, and chronic polyarthritis. Numbers of men and women studied were approximately equal.

TECHNIQUE

In all experiments determinations for ketone bodies were made according to the micro-method of J. E. Poulsen (1941). In all experiments 0.2 ml of blood was used, whereby the error of a single determination, as indicated by Poulsen, is $\varepsilon = \pm 0.4$ mg per cent. This figure is verified by the author.

The principle of the method is to convert the acetoacetic acid and, by means of potassium bichromatic and sulphuric acid, also the β-hydroxybutyric acid into acetone. In forming iodoform the latter binds a known quantity of iodine which is determined by titration wth sodium thiosulphate, starch being used as and indicator.

Contrary to most of the tests performed by Poulsen, I have made separate determinations for β-hydroxybutyric acid and acetoacetic acid + acetone. This procedure which, incidentally, is mentioned by Poulsen himself, yields more accurate results in determinations of "total ketone" in blood. In undergoing oxidation with potassium bichromate only about 69 per cent of the β-hydroxybutyric acid is converted into acetone. As this figure is fairly constant the total amount of β-hydroxybutyric acid can easily be computed when such a fractional determination is made. The joint estimation of all three kinds of ketone bodies yields less accurate results, the ratio of β-hydroxybutyric acid to total ketone being liable to some variation.

The total concentration of ketone bodies is expressed in terms of β-hydroxybutyric acid.

Determinations of blood sugar: method of Hagedorn & Norman Jensen, plasma chloride: Clausen's method, alkali reserve. method of Van Slyke & Neill, blood urea: method of Andersen & Lauritzen.

INANITION KETONEMIA

The preparatory diet administered to the experimental subjects was a standard diet — in which the relative proportions of fat, carbohydrate and protein prevented the occurrence, under any circumstances, of ketonaemia. The subjects were starved for a period of three days (the last test being performed at 8 a. m. on the fourth day). It was deemed unnecessary to continue inani-

tion beyond a three-day period as, according to earlier studies, the blood ketone concentration reached within this period will be maintained at a fairly constant level during continued inanition. (Wick, Sherrill & MacKay (1940), Gammeltoft (1944) and others). The subjects were given three glasses of water daily, which is important, as a deficient liquid intake will increase the inanition acidosis. During the inanition period the patients were confined to bed. A total of 25 persons were included in the study.

Determinations of blood ketone and blood sugar concentrations were made at 8 a. m., 2 p. m. and 8 p. m. on each of the three days and at 8 a. m. on the fourth day. At the same time the urinary output of ketone bodies was estimated by the tests of Gerhardt and Legal. Legal's test was positive in practically all instances of blood ketone concentrations of over about 10 mg%, expressed in terms of β-hydroxybutyric acid, and Gerhardt's test was positive in those cases in which the total ketone content of the blood

(also expressed in terms of β-hydroxybutyric acid) represented values ranging between 20 and 22 mg%. These findings are in good agreement with the observations of Poulsen (1941) according to which Legal's test was positive when the ketonaemia (expressed in terms of acetone) exceeded 5 mg% and Gerhardt's test was positive when the blood ketone concentration exceeded 11 mg%.

The experimental results are shown in Fig. 1.

It will be seen that the normal blood ketone concentration was very low, 3 mg% on the average, and never more than 5 mg% in persons who had ingested a meal (standard diet) 14 hours prior to the initiation of the experiment. There was a steady increase during the three day period and at the end of the experiment on the morning of the fourth day, the blood ketone concentration averaged 23.6 mg%. In order to illustrate the uniformity of the values, the patients were divided into three groups on the basis of the blood ketone concentrations estimated at 8 a. m. on the fourth day (Fig. 2).

It will be seen that the great majority (18 of 25 persons) had between 11 and 30 mg of β-hydroxybutyric acid in the blood.

There would appear to be an interrelationship between the blood sugar level at the end of the inanition period and the blood ketone concentration, the latter concentration increasing with a decrease in the blood sugar level (Fig. 3).

Determinations of blood urea, plasma bicarbonate and plasma chloride were made at the beginning and at the end of the experiments. In most cases but not in all, however) there was a moderate rise of blood
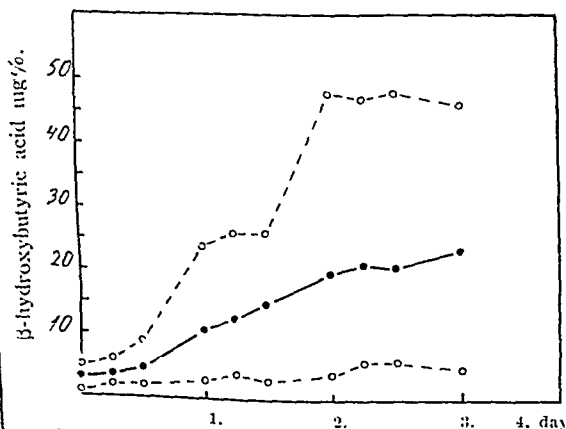
Fig. 1. The ketonemia during starvation (3 days). Dark line: Average values (25 indiv.). Stippled lines: highest and lowest findings.

25 Pt.
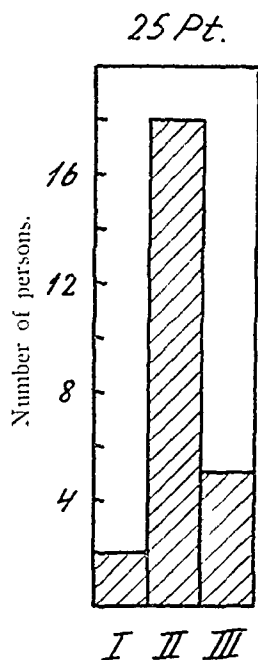
Fig. 2. The degree of ketonemia in normal persons after 3 days of starvation. The 25 persons are individed in 3 groups:

I: 0—10 mg% β-hydroxybutyric acid.
II: 11—30   »   —   —
III: > 30   »   —   —
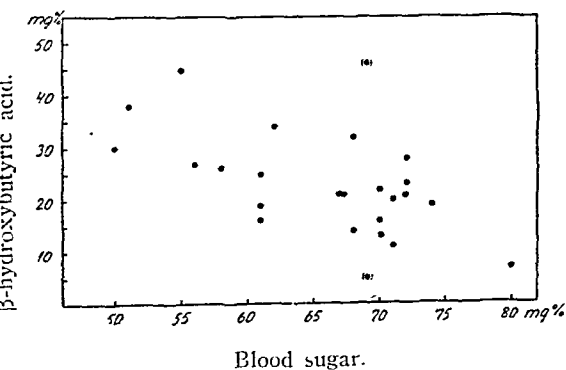
Blood sugar.

Fig. 3. The relation between blood sugar and ketonemia following 3 days of starvation.

urea. There was only a moderate drop of plasma bicarbonate. in some instances none at all.

The patients lost weight during the inanition period (generally between 1 and 2 kg). Immediately after the end of the experiment there was a weight increase of a few hundred g, even in those individuals who for experimental purposes were placed on a reducing diet (800 calories). This may possibly be explained by the fact that the patients lost some salt and water with the result that they put on weight even on an inadequate caloric intake, salt and water being retained in sufficient amounts to make up the deficit. It is important that this fact be brought to the notice of the patient who is placed on absolute diet for reducing purposes as, otherwise, he will fail to understand why he puts on weight on a reducing diet of 800 calories ingested subsequent to a period of fasting.

KETONAEMIA FOLLOWING KETOGENIC DIET

The subject serving in this series of experiments were all confined to bed. For preliminary period of 3 days they received a high fat diet of the following caloric distribution: fat 85 %, protein 10 % and carbohydrate 5 %. The diet provided about 1900 calories per day.

It will appear from the following that on this diet, the experimental subjects developed slight ketonaemia. During the three day period the blood ketone concentration rose at a fairly steady rate. (If the subjects were submitted to the ketogenic diet beyond this period, which I tried in a few instances, the diet here employed did not produce a further rise of the blood ketone level).

On the morning of the fourth day a cream tolerance test was performed. 250 ml of 36% cream were ingested.

At the initiation of the cream tolerance test the blood ketone concentration averaged 13.4 mg%. During the 6 hour period there was a steady rise to the average level of 22 mg%. On the basis of the blood ketone concentration obtained after three days on the ketogenic diet (i. e. at the initiation of the cream tolerance test on the morning of the fourth day) the patients may be divided into three groups:

The great majority of the experimental subjects (20 of 27) had a blood ketone level between 11 and 20 mg% (expressed in terms of β-hydroxybutyric acid), 6 persons between 0—10 mg% and 1 > 20 mg%.

The cream tolerance test with determination of blood ketone concentrations at hourly intervals was performed with a view to ascertaining, among other things, whether the rise of blood ketone concentration thus produced varied with the degree of ketonaemia observed prior to the initiation of the tolerance test. As might be expected it appeared that, on the average, the test ingestion of cream produced the greatest rise of blood ketone concentration in the patients showing the highest degrees of ketonaemia prior to the initiation of the test.

The blood ketone levels found both in the experiments with inanition and in the experiments with ketogenic diet are in good agreement with earlier investigations.

The present study of normal subjects had a twofold aim, 1) to determine, on the basis of a comparatively large number, of subjects, the maximum degree of ketonaemia attainable and 2) to obtain fairly reliable values permitting comparative studies of the rôle of ketone metabolism in health and in various pathological states.

SUMMARY

The blood ketone concentration obtained by inanition was determined on 25 normal subjects. In 3 days this concentration rose on an average from 3 mg% β-hydroxybutyric acid to 23.6 mg%, with rather considerable individual variation. Following 3 days' starvation two thirds of the experimental subjects (18 out of 25) showed a blood ketone concentration of between 11 mg% and 30 mg%. The highest blood ketone values were found in the persons showing the lowest blood sugar concentration at the end of the inanition period.

Alimentary ketonaemia was investigated in 27 normal subjects who, after 3 days on ketogenic diet, were subjected to a ketone tolerance test with ingestion of heavy cream, and then the blood ketone concentration was determined every hour for the following 6 hours. The results thus obtained appear to agree fairly well with those reported by most previous investigators. The increase in the concentration following the intake of the cream proved to be greater in the subjects showing a higher blood ketone concentration at the initiation of the test.

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KETONE METABOLISM IN HEALTH AND IN HEPATIC DISEASE

II. KETONE METABOLISM IN HEPATIC DISEASE

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When fat metabolism is induced, the blood ketone concentration will increase. This will be the case in carbohydrate inanition, and in phlorrhizin poisoning. Under these conditions the increase of fat metabolism is due to lack of carbohydrate resulting from deficient supply or increased excretion.

Ketonaemia also occurs in disturbances of the intermediary metabolism of carbohydrates. The most familiar example of this is diabetes mellitus. Considering the highly significant role of the liver in carbohydrate metabolism, it would be reasonable to assume that diseases of the liver may produce disturbances of the carbohydrate metabolism which, in turn, may influence the metabolism of fat.

In the following I shall first discuss the changes of carbohydrate metabolism in hepatic disease, and then the direct determinations of ketone bodies made on patients with hepatic disease.

The fasting blood sugar level is often slightly elevated. The assimilation limit for carbohydrate is frequently reduced, prolonged glucose tolerance curves and glycosuria being observable. Contrary to the assumption of earlier workers, the glycogen content of the liver is within normal limits (Krarup 1939). The liver glycogen is sup-posed to be .mobilized more sluggishly than in the normal person. All these changes are fairly characterictic of lesions of the parenchyma of the liver, whereas they are absent for example in cholelithiasis.

There is much difference of opinion about ketone metabolism in diseases of the liver. There has been a tendency to associate all disturbances of ketone metabolism with patological conditions in the liver. This was the case with the ketonaemia of pregnancy (Porges & Novak, 1911), the accumulation of ketone bodies during anaesthesia (Labbé, 1912) and with infectious disease and febrile conditions (Stroebe, 1931). In 7 patients Stroebe studied hepatic coma which ended fatally. The patients showed moderate degrees of ketonaemia. He also studied hepatic cirrhosis of a moderate degree in 8 patients. Only in some instances did he find moderate degrees of ketonaemia. Stroebe pointed out that there seemed to be some relationship between the clinical state and the degree of ketonaemia. Berlin & Jitz (1928) found that the urinary output of ketone bodies following the ingestion of a high fat diet was greater in patients with hepatic disease than in normal subjects. Contrary to this, several workers found a smaller degree of ketonaemia in hepatic damage.

Thus it is impossible, on the basis of previous studies, to acquire definite knowledge of the changes of ketone metabolism in hepatic disease, as the results are contradictory.

PERSONAL INVESTIGATION

The ketone metabolism in patients with hepatic disease was studied by the same methods and under exactly the same conditions as in the normal subjects, i. e. 1) by means of inanition and 2) by means of a cream tolerance test performed after 3 days on a ketogenic diet.

Like the normal persons, the patients were subjected to the following tests: Blood sugar analyses performed simultaneously with determinations of ketone bodies, the urinary tests of Gerhardt and Legal, and determinations of plasma chloride, plasma bicarbonate and blood urea made at the beginning and at the end of the experiment. The liver function tests included determinations of plasma colour index, the reaction of Takata, the galactose test and, in a number of instances, biopsy performed by Poul Iversen, M. D.).

INANITION KETONAEMIA

Experimental inanition was induced in 27 patients; 22 with epidemic hepatitis, 2 with chronic hepatitis and 3 with obstructive jaundice. The experimental conditions were identical with those under which the normal subjects were studied.

The curves were practically identical with those obtained in the inanition experiments with normal subjects. Like the normal subjects, the patients were divided into three groups on the basis of the blood ketone concentration estimated on the morning of the fourth day of inanition.

The great majority of the patients (17 of 27) showed blood ketone concentrations ranging between 11 and 30 mg%, and on the whole, there was no certain deviation from normal in the patients; the initial values, the rate of increase in concentration and the final values being almost identical with those observed in the normal subjects.

Liver biopsy performed at the end of the inanition period on 6 of the patients with moderately severe or severe inanition ketonaemia *revealed that the hepatic tissue was practically free of glycogen.*

Determinations of blood urea, alkali reserve and plasma chloride were made at the beginning and at the end of the experiment. The findings were in agreement with those obtained in the normal subjects. There was a moderate increase of blood urea or none at all, a moderate drop of alkali reserve and sometimes also a drop of plasma chloride.

With regard to the blood sugar level, I found that frequently the initial level was just above normal or actually increased.
The slightly elevated initial values did not seem to influence the formation of ketone bodies.
The drop in blood sugar was usually smaller in the normal subjects than in the patients with hepatic disease, the initial blood sugar level had no influence upon the magnitude of the drop. On the average, *the patients with hepatic disease experienced a greater drop in blood sugar and showed higher initial blood sugar levels.* Further, it appeared that the drop of blood sugar showed a definite tendency to be greatest in those who had the highest initial values.

In the normal subjects it was found that the degree of inanition ketonaemia increased with the decrease of the blood sugar concentration during inanition.

If there is any relationship at all between the ability of patients with hepatic disease to accumulate ketone bodies during inanition and their blood sugar level, it would appear that the patients with the lowest blood sugar concentrations at the end of the starvation show the highest degrees of ketonemia, whereas neither the blood sugar level nor the magnitude of the drop in blood sugar are of importance.

As mentioned previously, biopsy performed on some of the patients at the end of the inanition period revealed practically glycogen-free hepatic tissues. *Consequently, these patients are able to mobilize liver glycogen except,* as pointed out earlier in this report, in response to adrenaline injection.

Some workers (e. g. Hammarsten & Ståhle, 1943) assume that pancreatic lesions are responsible for the high fasting blood sugar and the prolonged sugar tolerance curves which are sometimes observed, and point out, in support of this view, that there are various indications of pancreatic lesions. That such lesions are present is, of course, indisputable. Hammarsten & Ståhle attach importance to the fact that the liver glycogen is frequently normal in patients with hepatic disease, and, therefore, they think that the high blood sugar concentration is not due to mobilization of liver glycogen.

In this connection it should be pointed out, however, that a histologic examination, including special glycogen staining of the tissue removed by biopsy, may reveal a normal glycogen content notwithstanding slight increase in the fasting blood sugar level.

It is true that an increase in the glucose content of the blood may give rise to increased consumption in the peripheral tissues, simply on account of the increased supply. The increases of glucose concentration are so slight, however, that the amount of glucose released in the liver will no doubt be so small that it will hardly be ascertainable by the comparatively gross method here employed for determination of the liver glycogen content.

KETONAEMIA FOLLOWING KETOGENIC DIET

Like the normal subjects, the patients were submitted to a cream tolerance test following 3 days on a ketogenic diet. A total of 26 patients served in this experiment; 24 with epidemic hepatitis, 1 with hepatic cirrhosis accompanied by ascites and incipient hepatargia and 1 with cholelithiasis.

There was a marked difference between the patients with hepatic disease and the normal subjects. The values obtained here were distinctly lower in the normal subjects. After 3 days on a ketogenic diet the blood ketone concentration on an average was 10.5 mg% β-hydroxybutyric acid (normal values: 13.4 mg%), and 6 hours after the ingestion of cream these liver patients showed on an average 17 mg%, while in the normal subjects the corresponding value was 22 mg%.

No less than 14 of 26 patients with hepatic disease had a blood ketone level ranging between 0 and 10 mg% after three days on

a ketogenic diet, whereas only 6 of 27 normal subjects exhibited such low concentrations under identical conditions.

As these experiments revealed that *the alimentary ketonaemia was far less pronounced in patients with hepatic disease than in normal subjects,* it was deemed appropriate to determine whether there was any relationship between various liver function tests and the blood ketone concentration following the ketogenic diet. Determination of plasma colour index and Takata-Ara reaction in the 14 patients with a concentration of 0—10 mg% of β-hydroxybutyric acid in the blood after 3 days on a ketogenic diet showed that there was no relation between the findings recorded above and the slight degree of alimentary ketonaemia.

Whereas the inanition experiments revealed that there was no difference with regard to the blood ketone concentration in normal subjects and in patients with hepatic disease, the experiments with a ketogenic diet showed clearly that the patients with hepatic disease often attained a lower blood ketone concentration than did the normal subjects.

There is hardly any doubt that *the slight degree of alimentary ketonaemia is related to the poor absorption of fats in these patients.* As pointed out previously, there is no essential difference between the ability of the liver to form ketone bodies from the fats contained in the food and from the body's own depots. Consequently, a more sluggish or incomplete absorption of fat in the patients with hepatic disease is the only explanation why these patients differ from normal subjects with regard to alimentary ketonaemia.

SUMMARY

Inanition ketonaemia was studied in a total of 27 patients with hepatic disease. These patients showed quite the same degrees of ketonaemia as were found in the normal subjects, with individual variations of practically the same magnitude. As demonstrated by previous workers, the *blood sugar* values were slightly increased in several instances. On the whole the fall in blood sugar concentration was greater in these patients than in the normal subjects, but the final values were about the same in both groups. In these patients too the highest degrees of inanition ketonaemia were found in the individuals showing the lowest blood sugar concentration at the end of the starvation period even though this feature here was less pronounced than in the normal subjects.

Liver biopsy was performed on some of the hepatic patients at the end of the starvation period. In all these instances the hepatic tissue was found to be practically glycogen-free, while in contrast hereto — as also demonstrated previously — the amount of liver glycogen was found to the normal on ordinary diet. Thus these patients are able to mobilize liver glycogen.

Alimentary ketonaemia was studied in 25 patients with hepatic lesions (epidemic hepa-

titis in 24, chronic hepatitis in 1, and cholelithiasis in 1). The values obtained here were distinctly lower than in the normal subjects. · As there is no fundamental difference in the ketonaemia developing under starvation and on a ketogenic diet, it seems justifiable to attribute the lower ketonaemia in the liver patients to incomplete or slow absorption from the intestinal tract. as the inanition ketonaemia is within normal limits in these patients.

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ICTERUS INDEX DETERMINED WITH THE ZEISS PULFRICH PHOTOMETER IN TURBID SERUM AND PLASMA[1]

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Photometric determination of the icterus index is more accurate than Meulengracht's dilution method and is always to be preferred when a photometer is available. Meulengracht's standard ought, however, to be maintained as in the method described by Waagstein (1946). He used a Zeiss Pulfrich photometer with the filter s47 and proved that light absorbed in icteric serum followed Lambert-Beer's laws.

When clear serum and water are compared in the photometer in identical cells, the extinction due to reflexion and refraction is the same and a pure absorptive extinction is obtained. With turbid serum, however, there is a considerable additional extinction from reflexion and refraction and the photometric method is consequently limited by the necessity of obtaining absolutely clear serum which must in consequence be taken before breakfast. But even when the sample is obtained before breakfast the serum may occasionally be turbid in such conditions as liver diseases. Plasma is always turbid. For that reason we wished to find a method to calculate the icterus index even in turbid serum and plasma.

A number of experiments were carried out with suspensions of finely precipitated barium sulphate, dilutions of normal and icteric sera and solutions of haemoglobin. Finely precipitated barium sulphate was suspended in water, a suitable concentration was chosen and dilutions made to 50, 25 and 12,5 %. The suspensions were placed in a Zeiss Pulfrich photometer and read against
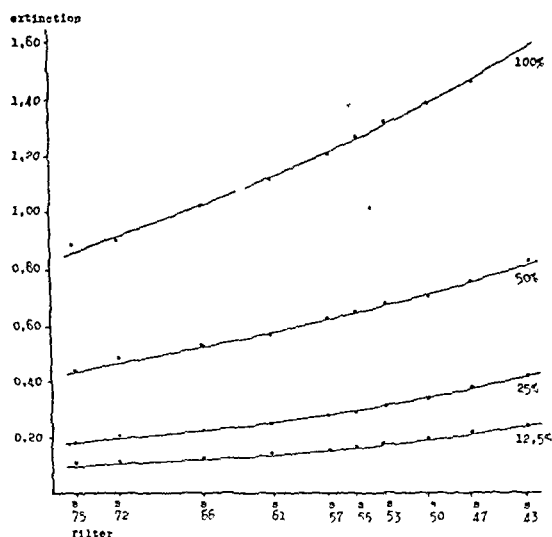
Fig. 1. Barium sulphate suspended in water and read against water in a Zeiss Pulfrich photometer.

water. The extinction curves plotted in Fig. 1 demonstrate that transmission is easier in the red than in the blue, which is a well known physical fact. The curves are slightly concave upwards but are approximately straight lines. Extinction read with one filter may then be calculated by reading two other filters (Fig. 2). In this experiment the extinctions are chiefly made up of reflexion and refraction and absorption is negligible. The extinction read with filter s47 is of special interest because this filter is used to determine the icterus index. In Fig. 2 two similar right-angled triangles are formed and the principal spectrum line of filter s61 is approximately halfway between

On account of turbidity the extinction with the filter s47 is

Fig. 2. The 50 % extinction curve drawn in Fig. 1.

those of filters s75 and s47. Each of the sides in the large triangle (ABC) is then twice as long as the corresponding side in the small triangle (ADE). BC is the difference beween the unknown extinction read with filter s47 and that with filter s75. DE is the difference between the extinction read with filter s61 and that with filter s75. On account of turbidity the extinction with filter s47 is: $2 E_{s61} \div E_{s75}$ (Fig. 2).

The extinction curves shown in Fig. 3 were read in normal serum (A), icteric serum (B) and a solution of haemoglobin

Fig. 3. Extinction curves read in normal serum (A), icteric serum (B) and a solution of haemoglobin (C). Read against water in a Zeiss Pulfrich photometer..

(C). None of them showed any extinction of significance with the two filters s75 and s61. These filters may then be used to calculate the extinction due to turbidity as described above (Fig. 2). Fig. 3 shows that haemolysis spoils the result in the photometric method as in the Meulengracht test. A considerable extinction is read with filter s47 in a solution of haemoglobin. The error introduced by haemolysis may be controlled, however, by reading one of the filters s57, s55 or s53. If the extinction read with any of these filters is distinctly different from that read with filter s61, haemoglobin or another coloured substance must be present in the serum.

To try to obtain physiological conditions as far as possible, icteric serum was diluted 1/10 with physiological saline and 1/10 with distilled water to produce a turbidity composed of a fine precipitate of globulins. Extinction curves are drawn in Fig. 4, Serum diluted with physiological saline (curve I) shows no significant extinction with filters s75 and s61. The serum diluted with distilled water yielded an extinction curve (curve II) which is elevated and shows an increment resembling that of the suspensions of barium sulphate. The extinction read with filter s47 was considerably different in the two curves (0.77 and 0.97). The extinction read with filter s47 corrected for turbidity is: $E_{s47} + E_{s75} \div 2 E_{s61}$ (Fig. 4).

Curve I: $E_{cor} = 0.77 + 0.05 \div 0.10 = 0.72$
Curve II: $E_{cor} = 0.97 + 0.13 \div 0.38 = 0.72$

It is usually sufficient to read each filter once and the icterus index is quickly calculated if the filters are read in the same succession as in the formula. The corrected extinction has to be multiplied by a factor to obtain the icterus index. This factor is the extinction coefficient read with filter s47 in Meulengracht's standard solution (0.12). The icterus index in Fig. 4 should then be 86 (i. e. $10 \times 12 \times 0.72$).

extinction

On account of turbidity the extinction with the filter s47 was·

$$2E_{s61} - E_{s75}$$

Extinction with the filter s47 corrected for turbidity

$$E_{s47} - (2E_{s61} - E_{s75})$$

$$E_{s47} cor. = E_{s47} + E_{s75} - 2E_{s61}$$

Fig. 4. Extinction curves read in icteric serum diluted 1/10 with physiological saline (I) and diluted 1/10 with distilled water (II). Read against water in a Zeiss Pulfrich photometer.

We recommend the use of undiluted serum in a 5 mm cell in non-icteric cases and in cases of jaundice serum diluted 1/10 with physiological saline in a 10 mm cell (occasionally a 5 mm cell with very heavy jaundice). The pure or diluted serum may be used for further investigations later on.

A pathological icterus index is always followed by a quantitative determination of bilirubin to avoid errors due to other coloured substances present in serum.

It is an obvious advantage to use the photometric method when a photometer is available. The dilution methods are the least accurate of all and, in addition, for the Meulengracht technique, daylight is desirable. There is no great difference in rapidity between the two methods. Waagstein (1946) considers that a sample from the ear lobe may suffice if microcells are available.

The Meulengracht test is surely the most suitable method in general practice, but for routine use in hospital the photometric method is preferable. The quantitative determination of bilirubin is complicated and is best reserved for control purposes.

SUMMARY

Extinction curves for various concentrations of barium sulphate suspended in water prove that transmission varies with the wavelength and that the extinction curves are approximately straight lines. Extinction read with one filter may then be computed by reading two other filters. Extinction read with filter s47 in turbid serum may then be corrected for turbidity by filters s75 and s61. $E_{cor.} = E_{s47} + E_{s75} - 2 E_{s61}$. The icterus index is calculated in accordance with Meulengracht's standard which gives the extinction $E_{s47}^{10mm} = 0.12$.

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MICROGASOMETRIC DETERMINATION OF CO₂
WITH THE SCHOLANDER-ROUGHTON SYRINGE-ANALYZER

WITH A COMPARISON OF ANALYSES CARRIED OUT BY THIS METHOD AND THAT OF VAN SLYKE AND NEILL

By V. FÜRST JR. AND O. MØRSTAD

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Scholander and Roughton have developed a method for CO₂ determination in approximately 13 cmm of full blood. As this method is very simple, quick and quite accurate, it should be suitable for the clinical laboratory. We have found the method very useful when working with newborn and infants, as well as small laboratory animals.

We have carried out approximately 130 single syringe analyses in 20 samples of full blood, 12 samples of plasma and 6 samples of capillary blood.

A. TECHNIQUE AND TECHNICAL COMMENTS

The description of the method is taken from the publications of Scholander et al. (1943, 1947) with small alterations. For further details we refer to the original papers.

The Principle

The principle is the same as that in Van Slyke's method. The sample is made acid, the gases extracted by evacuation and their volume read at atmospheric pressure before and after contact with alkali.

Apparatus

1. The Scholander-Roughton syringe analyser [1] (the micro gas burette) consists of a precision 0.5 mm Pyrex capillary fused to the nozzle of a 1 ml Pyrex tuberculin syringe which forms the extraction chamber (Fig. 1 a—d). The upper end of the capil-
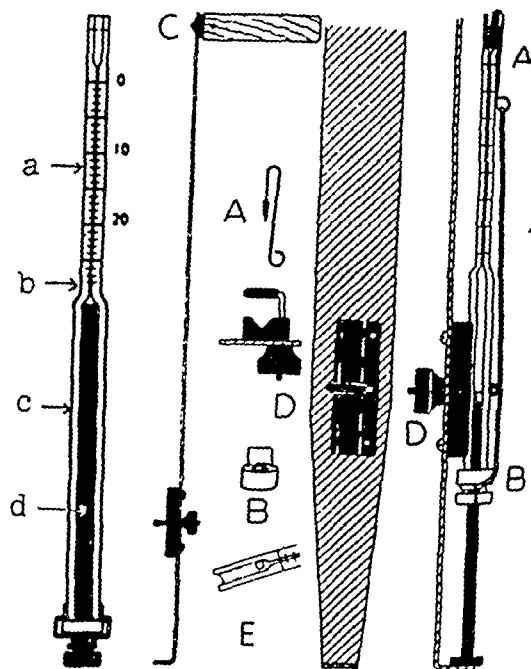
Fig. 1. The Scholander-Roughton syringe analyzer.
a) capillary. b) nozzle. c) barrel. d) plunger.
The hand sling and attachment.
A. plastic-tipped wire plug.   B. Spring steel clip.
C & D. Parts of the hand sling. E. The gas bubble during the CO₂ absorption.
(After Scholander, Flemister & Irving, J. Biol. Chem. 169. 173. 1947.)

lary is expanded to a cylindric cup and the main length of the capillary is graduated in 30 divisions, each of 2 mm length, with a total volume of approximately 13 cmm. The upper end of the capillary can be sealed with a plastic tipped wire plug (Fig. 1 A) pressed down into the cup by means of a rubber

band. During the vacuum extraction the plunger is arrested by a spring steel clip at the 0.8 ml mark Fig. 1 B).

2. A hand sling (Fig. 1 C—D) to which the Scholander-Roughton syringe can easily be attached, with the capillary pointing cen-tripetally for whirling down liquid bridges in the capillary and for breaking bubbles in the syringe.

3. As blood pipette, any slender, small pipette with ground tip can be used. As the blood is measured in the graduated capillary of the syringe analyzer, the pipette needs no calibration.

The blood sample is taken from a bleeding ear or finger prick. The pipette is filled by suction, the tip of the pipette is put deep into the blood drop so that no CO₂ can escape from the blood entering the pipette.

Reagents

1. Glycerol.
2. Caprylic alcohol.
3. Acid phosphate solution 95 g of $NaH_2PO_4 \cdot H_2O$ is dissolved in 100 ml of distilled water to make an almost saturated solution.
4. Distilled water freed from CO₂ by boiling with a drop of H_2SO_4.
5. 10 % NaOH.

Reagent 1 is best stored in a 2 ml syringe with a needle (size No. 20), the tip of which has been ground off square. The other reagents are stored in 2 or 5 ml all glass syringes with glass tips attached by rubber tubing or in small bottles with fine-tipped pipettes (Pasteur pipettes) with small rubber bulbs.

Procedure:

1. Remove the plunger from the syringe, rinse it well with very hot water, and dry by suction.
2. Lubricate the dry plunger with glycerol so no air is carried in with it. Avoid any glycerol entering the capillary.
3. Fill the dead space with distilled water, and remove the excess of water. Lower the

meniscus of the water 1 to 2 mm into the capillary.

4. With the blood pipette and syringe in nearly horisontal position, place the tip of the pipette firmly against the bottom of the cup, trapping a *small* air bubble between the blood and the water. Draw the blood *slowly and evenly* in to the 28 or 29 division mark. Remove the excess of blood and adjust the upper meniscus to the zero mark, and read the volume of the blood sample: *b divisions.*

5. Place a *small* drop of caprylic alcohol in the cup, expel the air bubble on the top of the blood, draw down 2 to 3 divisions of the alcohol and suck off the remainder. It is important to use as little alcohol as possible — although it must be enough to get rid of all the air bubbles in the blood mixture.

6. Fill the cup to the mark with acid phosphate solution without leaving any air bubbles in the capillary. Draw in the phosphate solution *slowly and evenly* until the meniscus is 2 mm down in the capillary.

7. Close the capillary with the plastic-tipped wire plug, trapping a small air bubble underneath. Add a drop of phosphate sol. to the cup to complete the vacuum seal.

8. Pull the plunger out *slowly and evenly* so that the capillary *drains properly* when the small bubble underneath the plug expands. Continue until the plunger notch engages the clip. Add *quickly* glycerol solution around the plunger bearing.

9. Attach the syringe to the sling as illustrated. Whirl the blood solution down and tilt it back and forth several times to mix the liquids well. Whirl the sling for a few seconds and tilt again until *all the bubbles have disappeared* or only traces are left. Whirl so for 15—30 seconds. The capillary should be clear of bridges.

10. 1½—2 min. after step 7 remove the syringe from the sling without touching the capillary. Raise the cup. Release the plunger stop and move the fluid *carefully* and *quickly* up into the capillary until atmospheric pressure is almost reached.

11. *Without delay* remove the plastic plug and adjust the upper gas meniscus at once exactly to the zero mark and read the volume (V_1) of the gas bubble. Do not move the gas bubble back and forth. Delay and fumbling in steps 10 and 11 will result in some reabsorption of CO_2.

12. Remove the acid phosphate solution from the cup, and fill it with NaOH. Invert the syringe (cup downwards), run the gas bubble out into the alkali (Fig. 1 E) and rotate a few times to facilitate absorption. Draw some alkali into the capillary and then the gas bubble. Measure its volume (V_2).

13. Remove the plunger gently from the barrel under the water faucet. Shake out the contents of the barrel and wash with *running tap water several times before the* plunger is again inserted. Dichromate cleaning solution should be used after each analysis to insure that no protein remains sticking to the capillary. While the syringe is still hot, dry it by suction.

Calculation

ml CO_2 in 100 ml blood $= (V_1 - V_2 - c)$ $\times f \times \dfrac{100}{b} \times i$.

$c =$ the reagents blank, approximately 0.1 division.

$f =$ the *correcting factor for temperature,* barometric pressure, and water vapor to dry volumes at $0°C$ and 760 mm Hg (see Peters and Van Slyke, 1932, p. 129, Table XV) (1932). As most of our analyses are done at 20—22° C we use a constant value: $f = 0.90$.

$i =$ the combined factor for the incomplete extraction and reabsorption of CO_2. Scholander et al. found an average value: 1.015, Lilienthal & Riley (1946) found: 1.023; we use approximately 1.02, so the formula comes out as $(V_1 - V_2 - 0.1) \times 0.90 \times \dfrac{100}{b}$ $\times 1.02 = \dfrac{(V_1 - (V_2 + 0.1)) \times 92}{b}.$

In this analysis there are two important points:

1. The capillary must be absolutely clean and free from solution bridges after the last whirling. When working with plasma taken under paraffin oil, great care should be taken to avoid letting paraffin droplets enter the capillary.

2. The readings must be accurate within 0.1 of a division. All the readings should therefore be made with a good lense. It is convenient to put the lense in a holder so both hands are free for the exact adjustment of the meniscus to the zero mark. A difference of 0.2 division in the blood volume and of 0.2 division in the opposite direction of the $V_1 - V_2$ volume, can give a difference in the CO_2 content of 1 vol %. Avoid errors due to parallax.

As the room air has a low CO_2 content, a *very small* leakage does no harm in this analysis. Good glycerol is therefore a sufficiently heavy lubricant.

To obtain duplicate analyses it is convenient to work with 2 syringes at a time. After lubrication and when the dead space has been filled with water, enough blood for two analyses, approximately 30 cmm, is taken into the transfer pipette, and both syringes are filled to the 28 mark. Proceed further with the last one, draw down the phosphate solution (step 6), mix it with the blood and lay the syringe aside. Repeat the same steps with the other syringe as soon as possible, and continue carrying this analysis through. Proceed further with the first syringe. To be quite sure not to mix up the syringe, write down their numbers together with the blood volume.

A duplicate analysis takes about 18 min. If one is working with 4 syringes and has an assistent to wash and lubricate the syringes, 8 to 10 analyses can be done in an hour.

B. INVESTIGATION ON THE METHOD OF SCHOLANDER-ROUGHTON, AND A COMPARISON OF THIS METHOD WITH THAT OF VAN SLYKE & NEILL

Since one of us needed a method for CO_2 determination in minute amounts of blood from small laboratory animals, and the other

Table I.
Total CO$_2$ in vol%.

Date	Full blood		Plasma		Difference between CO$_2$ in plasma and full blood.	
	1. Syringe method		1. Syringe method			
	Mean	Standard deviation	Mean	Standard deviation	Syringe method	Van Slyke's method
$^2/_7$	40.1 (4)	0.52	51.2 (4)	0.70	11.1	11.5
	41.8 (3)	2.15	50.2 (2)	2.35	8.4	8.5
$^3/_7$	46.5 (4)	1.77	53.0 (3)	0.54	6.5	11.4
	44.8 (4)	1.57	53.6 (3)	0.71	8.8	9.3
	40.2 (4)	1.04	47.5 (2)	0 07	7.3	6.2
$^4/_7$	50.9 (4)	0.73	57.6 (4)	1.37	6.7	7.4
	53.7 (3)	0.71	63.8 (4)	2.25	10.1	8.6
$^5/_7$	43.0 (2)	0.28	50.6 (2)	0.35	7.6	6.8
	43.5 (2)	0.35	48.4 (2)	0.28	4.9	6.5
$^3/_8$	59.4 (4)	1.46				
	43.6 (5)	0.65	48.6 (2)	0.07	5.0	7.7
$^8/_8$	38.9 (4)	0.82	46.5 (2)	0.14	7.6	7.5
$^9/_8$	42.3 (3)	0.51	48.1 (3)	0.68	5.8	8.0

The figures in brackets indicate number of duplicate determinations

needed a micro method for analyses of blood from newborn and infants, it was found desirable to compare the Scholander-Roughton method with an accepted method, and the Van Slyke & Neill was chosen as the most common. This comparison has been made with blood from human beings, — normal persons and pregnant women.

The blood was taken from a cubital vein into a 10 or 20 ml all glass syringe with the dead space filled with heparin. A drop of mercury was drawn into the syringe for mixing the blood. A small rubber tube was joined to the nozzle, filled with blood and closed with a glass rod, avoiding air bubbles. The syringe was kept in ice water. The pipette was filled directly from the syringe (Scholander & Roughton, 1943). After the CO$_2$ had been determined in the full blood by both methods, the rest of the blood was centrifuged under paraffin oil and the CO$_2$ determined in the plasma in the same manner.

The results of the analyses of the different samples, and the comparison between the two methods are shown in Table I. At the beginning there was some trouble with the method. A source of great error was the foam in the syringe after the vacuum extraction. To overcome this the amount of caprylic alcohol was increased, but this resulted in lower values. A blood sample free of foam, with the correct amount of caprylic alcohol (1—2 divisions), gave an average of 63.8 vol%, and with two big drops: 57.7 vol%. To clean the capillary thoroughly, the cup was filled to the top with phosphate solution and a CO$_2$ value of 63.8 vol% was obtained instead of 65.4 vol% when this solution was correctly filled only to the mark. When transferring samples from the blood syringe to the pipette, the tip of the pipette was put deep into the rubber tube joined to the nozzle and filled by pushing in the plunger. This analysis gave 63.6 vol%. When the pipette was filled by capillary attraction from hanging drops pressed out of the rubber tube, the analyses resulted in 60.6 and 58.9 vol%. All these analyses are duplicates.

As the difficulties were detected and overcome the results improved. Standard deviation greater than ± 1 vol% occured five

times in the 13 full blood analyses, and three times in the 12 plasma samples. The syringe method gave values which were mostly lower than those with Van Slyke's method, in average about 0.3 vol%.

As seen in Table I the total CO_2 content of full blood was from 5 to 11 vol% lower than that of plasma, with the mean for the syringe method 7.5 vol% and for Van Slyke's method 8.3 vol%. In 6 cases we also analyzed capillary full blood from the lobe of the ear. These values are still lower (mean 3.4 vol%) than full blood from the veins, as the first blood is nearer the arterial blood.

When the syringe method is used on human beings the samples will normally be taken from capillary blood. In these 12 samples we found a mean fullblood value of 8 vol% lower than the plasma value. In the 6 cases where also blood from the lobe of the ear was examined, we found a mean value 11.5 vol% lower than the plasma value.

Instead of trying to find a correlation factor between full blood and plasma values, and one between capillary and venous blood, we have commenced collecting normal values for capillary full blood from newborn and infants with a standardised technique.

CONCLUSION

1. The Scholander-Roughton syringe method gave, with some experience, a standard deviation of approximately 1.2 vol%. The values are usually slightly lower than those obtained with Van Slyke's method (mean 0.3 vol%).

2. When using the syringe method one must bear in mind that full blood gives lower CO_2 values than plasma, and capillary blood still lower figures.

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THE SCANDINAVIAN SYMPOSIUM ON THE KIDNEY IN HEALTH AND DISEASE 1949

A Scandinavian symposium on the kidney in health and disease was held in Copenhagen on September 2. to 4. 1949. The symposium was originally suggested by Hilding Berglund of Stockholm, who was the chief force behind the kidney symposium in Minneapolis in 1930.

The Copenhagen symposium was organized by members of the "Kidney Club" in that city, under the direction of Drs. J. Bing, T. Bjering and P. Iversen with P. Iversen as president. Some fifty papers were presented, covering normal and pathological anatomy, histochemistry, physiology and pathology.

The present number of The Scandinavian Journal of Clinical & Laboratory Investigation brings in extenso those papers which have not appeared or will not appear elsewhere, and which at the same time conform with the principles laid down for this periodical. The rest of the papers given at the symposium are presented here in extracts furnished by the authors. A complete list of all papers delivered at the symposium will also be found.

LIST OF PAPERS

Sept. 2, A. M.

G. GLIMSTEDT: Structure and histochemistry of the nephron.

*E. LJUNGBERG: Chlorides in the kidney, the blood and the urine in experimental nephritis.

B. VIMTRUP: Histological examinations of kidneys of Heteromyidae.

K. KOEFOED: Investigation of the kidneys of the Kangaroo rat by the maceration-method.

J. BING and G. TEILUM: Effect of intraperitoneal injections of protein hydrolysates on rat kidneys.

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* Indicates papers published in extenso. All the others are abstracts.

O. Torgersen: Further studies on the morphology of pre-urine in situ.

F. Löfgren: The topographical arrangement of the malpighian pyramids in the human kidney.

*A. Bergstrand and H. Bergstrand: The pathology of glomerulonephritis and related diseases.

M. Simonsen: Endocrine kidney.

Sept. 2, P. M.

T. Bjering: a. Measurement of glomerulae filtration rate. b. Renal excretion of urea.

E. Blegen, H. N. Haugen and K. Aas: Endogenous "creatinine" clearance.

P. Effersøe: Comparative investigations into the inulin, creatinine and thiosulphate clearances of rabbits after ingesting sodium benzoate.

*H. Bucht: On the tubular excretion of thiosulphate and creatinine under the influence of caronamide.

P. Effersøe: On the effects of filtration and concentration on the backdiffusion of urea in the tubules of rabbits.

C. Brun: The influence of renal function tests on the function of the kidneys.

B. Josephson: The mechanism of renal tubular excretion.

*H. Bucht, L. Werkö and B. Josephson: The oxygen consumption of the human kidney during heavy tubular excretory work.

F. Tudvad and J. Vesterdal: Inulin and PAH clearances in newborn infants.

I. Sperber: The excretion of some organic bases and some phenols and phenol derivatives.

P. Effersøe: Comparative determinations of the creatinine and thiosulphate clearances in kittens.

E. Bojesen: Secretion of diodrast by the small intestine.

Sept. 3, A. M.

*F. Tudvad: Sugar reabsorption in prematures and full term babies.

K. Lundbæk and V. Posborg Petersen: Filtration and glucose T_m in late diabetes mellitus.

*T. Hilden: Glomerular filtration rate and maximum tubular excretory capacity in diabetic nephropathy.

S. L. Sveinsson: Renal excretion of glycerol.

M. Bjørneboe, S. Dalgaard-Mikkelsen and F. Raaschou: Does the filtration (thiosulphate clearance) differ at different pH values in the urine?

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* Indicates papers published in extenso. All the others are abstracts.

*M. Bjørneboe, S. Dalgaard-Mikkelsen and F. Raaschou: On the excretion of salicylic acid in man. (A preliminary report).

*E. Bojesen: The function of the urinary tract as "dead space" in clearance experiments. (A preliminary report).

C. Brun, T. Hilden and F. Raaschou: Examinations of the delay-time of the kidney.

*A. Levin Nielsen and H. O. Bang: The protein content of the diet and the function of the kidneys in human beings.

Sept. 3, P. M.

T. Hilden: On the renal mechanism for excretion of water and salt.

*E. Blegen: Kidney function in heart failure.

*T. Hilden: Glomerular filtration rate and maximal tubular excretory capacity in congestive heart failure.

H. Eder: The mechanism of nephrotic edema.

E. Møller-Christensen: Diuresis of rats after peroral ingestion of water in massive doses.

O. J. Malm: Studies on the recovery of renal function after resection on the single kidney in renal tuberculosis.

O. Povlsen: Bladder-neck obstruction; back pressure; kidney function.

M. Simonsen: Kidney transplantations.

A. Levin Nielsen, K. Trautner and J. Moustgård: Kidney transplantations.

*A. Palmlöv: Some observations on the Trueta renal vascular shunt.

*L. Werkö, H. Bucht and B. Josephson: The renal extraction of para-aminohippuric acid and oxygen in man during postural changes of the circulation.

Sept. 4, A. M.

P. Hedlund: The pathogenesis of glomerulonephritis.

J. Rudebeck: Prognosis of acute glomerulonephritis.

N. Alwall: Treatment of uremia with artificial kidney.

*C. Brun: On the treatment of uræmia with intestinal dialysis.

* Indicates papers published in extenso. All the others are abstracts.

CHLORIDES IN THE KIDNEY, THE BLOOD AND THE URINE IN EXPERIMENTAL NEPHRITIS

BY E. LJUNGBERG

From the Histological Iinstitute, University of Lund, and The Medical Department of the General Community Hospital, Malmö

Grünwald (1909) found that the chloride concentration in the kidney parenchyma of rabbits was higher in the medulla than in the cortex. In 1942 Glimstedt was able to show that this concentration was higher the closer to the kidney papilla the samples were taken. He considered the central increase of the chlorides to be due to an augmentation — an active reabsorption — of chlorides in certain cells of the collecting ducts. Ljungberg (1947) found that the chloride concentration in the central zone of the medulla could reach and even exceed that of whole blood.

As it might be of interest to study the active reabsorption of chlorides in nephritis, an investigation was undertaken with the object of examining the kidney chlorides in experimental Masugi nephritis in rabbits. This paper is a preliminary report of some of the results of this investigation.

METHODS

Chlorides were quantitatively determined in kidney tissue, whole blood and urine of 16 normal rabbits and 34 rabbits with experimental (Masugi) nephritis. All the animals with Masugi nephritis were given 3 ml of the same nephrotoxic plasma in one single injection. The plasma had been produced by immunization of 7 ducks with rabbit kidney. 2—3 days after the injection all the animals showed elevated blood pressure. 7 animals were killed and examined before they developed proteinuria. The rest, or 27 animals developed proteinuria a good 8 days after the injection. 16 animals were killed in this phase of the nephritis, while the remaining 11 animals, that had proteinuria for an average of 21 days, were killed and examined when they had recovered.

Chloride determinations in renal tissue were made according to Glimstedt, and in blood and urine according to Bang. As soon as the animals had been killed, samples of heart blood and bladder urine were taken. While the animals were alive, numerous determinations were made of the chloride concentration in urine (per diem) and in whole blood.

RESULTS

Fig. 1 shows the standard curve of chloride concentration in the kidneys of 16 normal rabbits. With Glimstedt's technique one reaches a good 10 mm down into the kidney, counted from the surface of the cortex; one reaches into the inner zone of the medulla, which of the whole tubular system of the kidney contains only Henle's thin loop and collecting ducts. To the apex of the papilla there is a distance of another 4—5 mm. — A ravine-like fall, marking the boundary between the cortex and the medulla, devides the curve in two main parts: one lower, plateau-like, cortical, and one higher, rising, medullar. The hillshaped rise in the first part corresponds to an accumulation of large
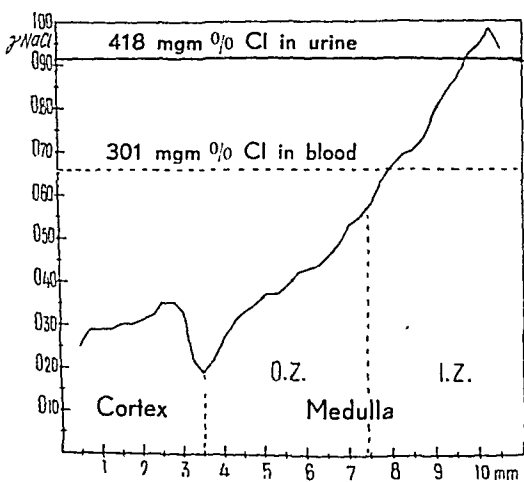
Fig. 1. The standard normal curve. x = depth of punching in mm. γ NaCl per section of a diameter of 3 mm and of a thickness of 0.01875 mm. Each point of the curve represents the mean value from chloride determinations of the same depth of 16 normal kidneys. The chloride concentration in urine and blood are drawn as a comparison.

tration in whole blood and even surpassed the chloride concentration in the urine.

Fig. 2 shows the standard curve of the 16 animals that were killed and examined when they had proteinuria. This curve is more irregular and considerably lower than a normal standard curve. The fall was greatest in the medullar part. The boundary between the cortex and the medulla was less distinct. In no layer of the kidney did the chloride concentration reach that of blood or urine. For the sake of comparison the normal curve is drawn as a dotted line.

The 7 animals that were examined before the occurrence of proteinuria already had a fall of the chloride curve in the kidney, while the 11 animals that were examined after the cessation of the proteinuria showed normal or slightly depressed chloride curves.

Fig. 3 shows the relation between the level of chlorides in blood and in urine in

vessels on this level. The standard normal curve can be said to represent the level of chlorides in different layers of the kidney. In the cortex the average chloride concentration was 142 mgm%. In the medulla the chloride concentration rose and 10.25 mm from the surface of the cortex it reached 449 mgm% (in the beginning of the inner zone of the medulla).

This figure also shows the level of chlorides in blood and urine in comparison to that in different layers of the kidney. In the cortex the chloride concentration was scarcely half that of whole blood. In the medulla the chloride concentration of the tissue rose and 8 mm from the surface of the cortex, i. e. in the inner zone of the medulla, it reached and exceeded the chloride concen-

Fig. 2. ———— The standard curve of 16 kidneys with nephritis during proteinuria.
.... The standard normal curve.
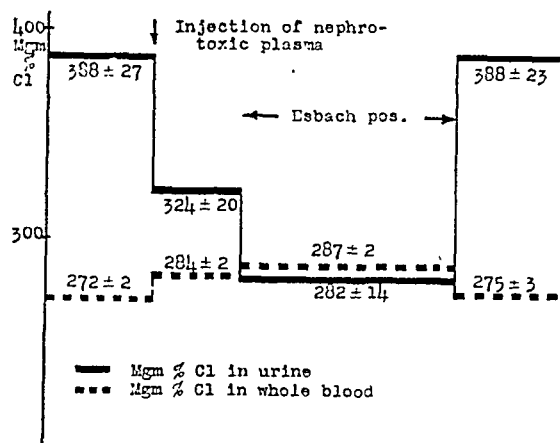
Fig. 3. The relation between the chloride level in urine and whole blood in different phases of nephritis.

different phases of the nephritis. The 34 animals with nephritis were examined the days before and after the injection of nephrotoxic plasma. During the phase with proteinuria 27 animals were examined and after that phase 11 animals. Each average is founded on 70—80 determinations. The diagram shows that the level of the chloride concentration in urine was more or less a reflection of that in the blood. The days after the injection of nephrotoxic plasma a fall of the level of chlorides in the urine was observed and at the same time a rise of the blood level. When the animals had developed proteinuria, the chloride level fell still more in urine and rose in blood. When the proteinuria ceased there was a return to almost the same values as before the injection.

CONCLUSIONS

In normal rabbits the chloride concentration in different layers of the kidneys was found to rise in the direction cortex-

medulla, the concentration in the cortex being about half as high as that in the blood. In the medulla the concentration rose in proportion to the distance from the surface of the cortex; about 8 mm from this the chloride concentration reached and exceeded the level of chlorides in blood and about 10 mm from the surface of the cortex it reached the level of chlorides in the urine.

In Masugi nephritis:

1) There was a fall in the chloride level both in the cortex and even more in the medulla; and the chloride concentration reached neither that of the blood nor that of the urine.

2) This fall of the chloride concentration came early (before the proteinuria) and it was reversible to a certain extent.

3) The fall of the chloride concentration in the kidney ran parallel with the fall of the chlorides in the urine and was also a reflection of the level of chlorides in the blood.

The decrease of the kidney parenchyma chlorides in experimental nephritis with proteinuria may be explained by a deterioration of the active reabsorption of chlorides. But it may also be due to a decreased glomerular filtration resulting in a decrease of the chloride supply to be reabsorbed by the tubular cells. This question will be discussed further in a paper which will be published later.

SUMMARY

Using Glimstedt's technique, the chloride concentration of rabbit kidney parenchyma was determined. In normal animals the concentration was higher in the medulla than in the cortex, with a gradual but steep

increase towards the central parts of the kidney where it even exceeded the concentration in the urine. In experimental nephritis the chloride concentration in the kidney was much lower than normal. This was especially the case in the medulla. The decrease appeared earlier than the proteinuria and contrasted to the increased chloride concentration in the blood. The decrease was reversible.

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ON THE TUBULAR EXCRETION OF THIOSULPHATE AND CREATININE UNDER THE INFLUENCE OF CARONAMIDE

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Caronamide (4-carboxy-phenyl-methan-sulfanilide) was synthesized in order to obtain a substance with the property to blockade the tubular excretion of penicillin in the kidneys. It has been clinically and pharmacologically tested mainly by Beyer and co-workers (1947, a, b, c).

At a Caronamide concentration in the plasma of about 15 mg per cent the renal tubular excretion of such substances as penicillin and para-amino-hippuric acid (PAH) was maximally retarded or obstructed (Boger 1947). This obstruction was reversible. On the other hand, Caronamide was of no influence on the back-resorbtion of e. g. glucose, urea or amino-acids (Beyer and co-workers 1947 b). Further Beyer and co-workers (1947 a) could not find any influence of Caronamide on the glomerular filtration or on the renal blood flow in dogs.

Since Rehberg (1926) in his examination of the creatinine excretion found that the creatinine clearance is very similar to the glomerular filtration, this clearance has been extensively used clinically as well as in physiological experiments. However, Shannon and co-workers (1935, 1940, 1941) could show that creatinine is also to some extent excreted tubularly in man. Their investigations have been confirmed by Smith and co-workers (1937). However, the tubular excretion of creatinine has been subject to discussions and especially Ekehorn (1944) raised several objections against the view that creatinine can be excreted by the tubular cells. Crawford (1948) and others have published objections against Ekehorn's interpretation. But it also seems as if the creatinine can be resorbed from the tubuli (Shannon and Winton 1940, Steinitz and Türkand 1940, Josephson and Lindahl 1945).

Several investigators have found a good conformity between the clearance of thiosulphate and that of inulin (Newman 1946, Gilman 1946, Pitts and Lotspeich 1947, Crawford 1948, Brun 1948, Langeron and co-workers 1949 and others). In this hospital we have not been able to find such a good conformity between these two clearances. The reason for this discrepancy is probably that we have used lower thiosulphate concentrations in plasma than other investigators. This reason will be further discussed later on. In this connection it may be mentioned that Bing and Effersöe (1948) showed that kidneys of cats have the ability to excrete thiosulphate tubularly. A priori it may seem unlikely that such a small molecule as that

of thiosulphate is filtered only and neither excreted nor reabsorbed in the tubuli.

The investigation reported here was undertaken in order to study the mechanism of the renal excretion of inulin, thiosulphate and creatinine. I have made use of the influence of Caronamide on the renal tubular function in this connection.

METHODS

Eight healthy human subjects were examined in 21 different experiments. Inulin, thiosulphate and creatinine clearances were determined simultaneously on all the subjects. When the experiments were undertaken the subjects· were in the postabsorptive state except that they had been given 6 g creatinine and about 1 l water by mouth about 1 hour before the experiment was started. In seven experiments the thiosulphate and the inulin were given intravenously in one single injection each. Usually 100 ml of 16 per cent sodium thiosulphate solution and 100 ml of a 10 per cent inulin solution were given but the amounts were varied according to the size of the subject and the concentrations wanted. In 14 experiments they were given in a slow constant injection with deliberately, slowly decreasing or increasing concentration. The thiosulphate concentrations in the plasma were deliberately varied between 1.5 and 113 mg per cent. The creatinine concentrations were rather constant between 5 and 8 mg per cent in all of the experiments.

Usually each experiment consisted of three clearance periods of about 20 minutes each with a variation of 1—2 minutes. The urine bladder was emptied by an indwelling catheter and after each period rinsed twice with 20 ml saline each time. The blood samples were taken at the start and at the end of each period by an indwelling needle in one of the brachial arteries (except in one case when only venous blood was taken). Inulin was analyzed according to Josephson and Godin's modification (1943) of Corcoran and Page's method (1939). Thiosulphate was analyzed according to Brun's (1948) modification of Newman and co-workers' method (1946). In the creatinine determinations the Folin method and a photo-

electric colorimeter were used. In separate experiments I have controlled that the presence of Caronamide is of no influence on the results of inulin, thiosulphate and creatinine determinations.

The three clearances were determined without Caronamide in the first experiment on each of the subjects. In some instances this "blank" determination was repeated later on. Some days after the first experiment the clearance examinations were repeated after the subject had been given Caronamide.[1] The Caronamide administration in 0.5 g tablets given by mouth was started some hours. before the experiment and the doses varied between 2 g every 4th hour and 4 g every 3rd hour. Caronamide was determined in plasma in three experiments in which the subjects were given the larger Caronamide dose. Collin and Finland's modification (1949) of Ziegler and Sprague's (1948) colorimetric method was used. In these cases the plasma concentrations were about 12 mg per cent. As the Caronamide concentration in the blood usually decreases rather rapidly after the administration, the blockade sometimes disappeared slowly during the experiment. In one of the experiments the clearance of Caronamide was determined. It was found to be 68 per cent of that of inulin.

RESULTS

Fig. 1 demonstrates the proportion be-, tween thiosulphate clearance and inulin clearance and between creatinine clearance and inulin clearance in three different experiments on one of the subjects. In the first experiment no Caronamide was given but in the subsequent experiments the subject was treated with different amounts of Caronamide. In the first experiment — that without Caronamide — the creatinine clearance was higher than that of thiosulphate. Both clearances are considerably higher than that of inulin. At the start of the second
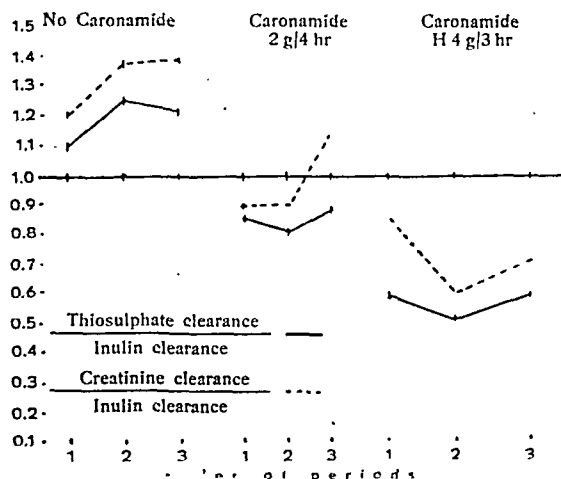
Fig. 1. The relations Thiosulphate/Inulinclearance and Creatinine/Inulinclearance in a helthy man with Caronamide and after different doses of Caronamide.

blocking effect was still more accentuated. Here the proportion between thiosulphate and inulin clearance was depressed as far as to about 0.5 and the proportion creatinine clearance inuline clearance to about 0.6. The Caronamide effect was evident during the whole experiment. In these three experiments the inulin and the thiosulphate were given as a slow intravenous injection. The plasma thiosulphate concentrations were rather constant at about 18 mg per cent.

In Table I the whole material is presented. In the statistical calculation the average clearance figure of each subject has been used. As the differences between the duration of the different periods were very small,

Table I.

	Without Caronamide		With Caronamide		With—Without Caronamide	
	average	σ	avarage	σ	average	σ
Inulin clearance	134 ± 8.3	23.6	157 ± 7.8	21.9	+ 22.5 ± 8.3	22
Thiosulphate clearance	181 ± 17.1	48.5	125 ± 4.3	12.2	− 55.9 ± 15.7	41.7
Creatinine clearance .	166 ± 2.9	8.2	155 ± 8.8	24.9	− 10.5 ± 7.0	19.7
Thiosulphate clear. / Inulin clear. ..	1.36 ± 0.10	0.29	0.81 ± 0.04	0.11	− 0.54 ± 0.11	0.30
Creatinine clear. / Inulin clear.	1.24 ± 0.03	0.07	0.99 ± 0.04	0.12	− 0.24 ± 0.04	0.12

Inulin clearance, Thiosulphate clearance and Creatinine clearance, the relation Thiosulphate/Inulin clearance and Creatinine/Inulin clearance with and without Caronamide.

experiment, that in which the subject had received the smaller Caronamide dose, the clearances of creatinine as well as of thiosulphate were lower than that of inulin. During the last period of this experiment the creatinine clearance rose above that of inulin. This rise was probably due to a decrease of the Caronamide concentration with a yielding tubular blockade as the result. In the third experiment — with the highest Caronamide dose — the tubular

they have not been taken into consideration at the calculations of the averages between the clearance periods.

The standard deviation has been calculated according to the formula $\sigma = \sqrt{\dfrac{\varepsilon\,d^2}{n-1}}$ and the standard error of the average according to the formula $\varepsilon\,(M) = \dfrac{\sigma}{\sqrt{n^2}}$. In these formulae σ = standard deviation, $\varepsilon(M)$ = average error of the average, d = the individual differences from the average and n = number of experiments.

Table II.

P_{Th}	$\dfrac{Th\ Cl}{In\ Cl}$	Number of periods
< 10	1.68	10
10 — 20	1.34	15
20 — 35	1.18	9
< 35	0.76	4

verage of $\dfrac{\text{Thiosulphate clearance}}{\text{Inulin clearance}} \left(\dfrac{Th\ Cl}{In\ Cl} \right)$ at different

inges of Thiosulphate concentration (P_{Th}) in plasma.

'he relation Thiosulphate/Inulin clearance at dif-
:rent plasmaconcentrations of Thiosulphate (with-
out Caronamide).

Inulin clearance was increased after Caro-
iamide. Statistically this increase is pro-
)able but not definitely proved. The decrease
)f the creatinine clearance after Caronamide
)n the other hand has no statistical support
ind is probably only a coincidence. On the
)ther hand the proportion between creati-
nine clearance and inulin clearance is signi-
ficantly diminished after Caronamide. At
the present it cannot be demonstrated if this
is due to an increase of the inulin clearance
or to a decrease of the creatinine clearance
or — which appears most likely — to a
combination of both these causes. That
question will be subject to further investig-
ations. The standard deviation of the crea-
tinine clearances is with statistical probabi-
lity increased after Caronamide.

The fact that the thiosulphate clearance
without Caronamide was higher than the
inulin clearance is statistically proved. With
Caronamide the thiosulphate clearance is
statistically significantly decreased and also
its standard deviation is decreased with sta-
tistical significance. Even with respect to
the change of the clearance figure this de-

crease of the standard deviation indicates
that the deviation obtained without Carona-
mide was not due to methodical errors.
Also the proportion thiosulphate clearance/
inulin clearance was depressed by Carona-
mide with statistical significance. After the
Caronamide administration this proportion
was proved to be below 1.0.

Table II demonstrates the proportion
between the thiosulphate clearance and the
inuline clearance at different plasma concen-
trations of thiosulphate. This proportion is
lower at high thiosulphate concentrations
than when the plasma concentration of thio-
sulphate was low.

DISCUSSION

The inulin clearance was increased when
Caronamide was given, and this increase is
statistically very probable, not far from
proved. This increase might be explained
by a Caronamide blockade of a hypothetical
tubular backresorbtion of inulin (Ekehorn
1944). However, this explanation seems
very unlikely as Caronamide has no in-
fluence on the back-resorbtion of other sub-
stances, such as glucose etc. Besides that,
a back-resorbtion in the tubuli of such a
large molecule as inulin does not seem
probable. Another hypothetical explanation
of the enhanced inulin clearance might be
that the Caronamide has increased the per-
meability of the tubular walls resulting in a
tubular secretion of inulin. This explanation
too seems very improbable as other carbo-
hydrates with small molecules, such as glu-
cose, do not pass that way and Caronamide
does not give glucosuria. It also seems un-
likely that inulin could be led through the
tubular wall into the lumen when an excre-

tion of substances known to prefer that way is hampered by Caronamide. The most probable explanation of the increased inulin clearance seems to be an augmentation of the glomerular filtration, eventually due to an augmented renal plasma flow.

The fact that Caronamide depresses the clearance of thiosulphate proves that thiosulphate can be tubularly excreted. A further indication that such an excretion takes place is the fact that the standard deviation of the figures for the thiosulphate clearance was depressed by Caronamide. The best explanation of this observation is that the deviation without Caronamide is due to variations in the tubular excretion. When the tubular excretion was blocked the variations were smaller.

Another proof of the tubular excretion of thiosulphate is the relation between the proportion thiosulphate clearance/inulin clearance and the plasma thiosulphate concentration. If only those experiments are taken into consideration in which no Caronamide was given the proportion thiosulphate clearance/inulin clearance was lower when the plasma thiosulphate was high and vice versa, although there were some exceptions. This shift of the proportion is probably due to a selfdepression of the thiosulphate clearance at increasing plasma concentrations. The fact that the thiosulphate clearance in the present material usually was found to be higher than the inulin clearance might be explained by the comparatively low concentrations of thiosulphate used. In more than ⅔ of the experiments the plasma thiosulphate concentration was below 20 mg per cent. Those previous investigators who have found a good conformity between the thiosulphate

and the inulin clearance have usually used higher thiosulphate concentrations, and for that reason they may have brought about a certain degree of self depression of the thiosulphate clearance.

But the results reported here do not only prove that thiosulphate is excreted by the tubuli. They also speak strongly in favour of a tubular back resorbtion of thiosulphate. Such a resorbtion is indicated by the fact that when Caronamide was given, the proportion thiosulphate clearance/inulin clearance was significantly lower than 1.0. This is illustrated e. g. by the experiment demonstrated in Fig. 1. When the tubular excretion of thiosulphate is blockaded by Caronamide, the tubular resorbtion is revealed.

As thiosulphate thus seems to be both excreted and resorbed in the tubuli it is not very astonishing that its clearance may turn out to be in conformity with the inulin clearance, but it is undoubtedly strange that several investigators have found such a good coincidence. The fact that a substance has a clearance identical with that of inulin is of course no proof that it is excreted by glomerular filtration only. A similarity with the inulin clearance could be achieved by a combination of factors retarding (backresorbtion, protein binding) and accelerating (tubular excretion) its output. Thus, e. g. Lindahl and Josephson (1945) found that sulfamethyl-thiodiazol has a clearance which is practically identical with that of inulin due to the fact that it is partly protein-bound in the plasma and partly excreted by the tubuli and that these properties counterbalance each other.

The proportion creatinine clearance/inulin clearance was found to be 1.24 ± 0.03 without Caronamide. This is in conformity with

the figures published by Homer Smith (1943) and others. Under the influence of Caronamide this proportion was diminished with statistical significance. This fact may be due either to an increase of the glomerular filtration or to a depression of the tubular excretion of creatinine. It seems most probable that the decrease of the proportion is due to both these causes. A tubular excretion of creatinine is also indicated by the increased standard deviation of the creatinine clearance when the subjects were under the influence of Caronamide.

It may seem illogical that the *increase* of the standard deviation of creatinine under Caronamide influence is taken as an indication of tubular excretion while a *decrease* of the corresponding deviation of the thiosulphate clearance after Caronamide is used as an argument for the tubular excretion of thiosulphate. However the depression limit for thiosulphate seems to be rather low and to vary individually and for that reason the deviation of its clearance figures gets comparatively high. Its tubular excretion seems to be rather easy to hamper with Caronamide and for that reason the deviation of its clearance decreases when Caronamide is given. The depression limit of creatinine on the other hand is high (Shannon 1940) and in all the experiments the creatinine concentration was well below that limit. For that reason creatinine clearance and inulin clearance run rather parallel to each other with a comparatively low deviation. Caronamide hampers the tubular excretion of creatinine to a moderate degree with individually varying sensitivity. For that reason it increases the deviation of its clearance.

The tubular reabsorbtion of creatinine, on the other hand, is demonstrated in some

of the experiments, where the tubular blockade has been more complete (Fig. 1). In some of these experiments the creatinine clearance became lower than that of the inulin, but in many experiments this shift in the proportion did not occur.

SUMMARY

Inulin, thiosulphate and creatinine clearances were determined simultaneously in 8 healthy human subjects. The determinations were repeated when the subjects had been given Caronamide in order to blockade the renal tubular excretion. The results show that thiosulphate is excreted as well as backresorbed in the tubuli. Tubular excretion and resorbtion of creatinine could not be statistically proved, but the results speak in favour of an excretion as well as a resorbtion. It also seems as if Caronamide is able to increase the glomerular filtration with an increased inulin clearance as the result.

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THE OXYGEN CONSUMPTION OF THE HUMAN KIDNEY DURING HEAVY TUBULAR EXCRETORY WORK

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It has long been known that the kidneys consume less oxygen than many other tissues. In experiments on dogs it was shown by Van Slyke, Rhoads, Miller and Alving (1934) that an increase of the urea output did not increase the oxygen consumption of the kidneys. This was confirmed by Eggleton, Pappenheimer and Winton (1940) who found that even a very considerable augmentation of diuresis, osmotic work, urea excretion and tubular resorbtion of urea were of very little influence on renal blood flow and renal oxygen consumption in dogs. According to these authors the kidney answers the demand for increased work not by increased metabolism but by increased working efficiency. These authors, however, found that an increased blood pressure followed by an increase of the glomerular filtration caused a rise of the oxygen consumption of the kidneys. On the other hand Dole, Emerson, Phillips, Hamilton and Van Slyke (1946) could not bring about an increase of the renal oxygen consumption in dogs when they elevated the blood pressure and the glomerular filtration by means of adrenalin, but they found the oxygen consumption to be rather parallel to the renal blood flow.

In man the oxygen consumption of the kidneys was first examined by Warren, Brannon and Merill (1944) using renal vein catheterization. They found that in man too the arterio-venous oxygen difference was less in the kidneys (2—3 ml per 100 ml blood) than in mixed blood taken from the heart (3.5—6 ml per 100 ml blood). Bradley and Halperin (1948) demonstrated the close connection between renal blood flow and oxygen consumption during abdominal compression in man. Reubi, Schroeder and Williams (1948) examined the renal oxygen consumption in man and found this consumption to decrease after an injection of adrenalin in spite of the fact that the glomerular filtration probably had risen.

Thus previous investigators have found the renal oxygen consumption to be comparatively low and attempts to increase this consumption by increasing the work of the kidneys seem to have failed. An increase of the diuresis, the osmotic work and the reabsorbtion of urea seem to be without effect. The effect on the oxygen consumption of tubular *excretory* work, however, does not seem to have been examined. If any, this kind of renal work could be expected to cause an increase of the oxygen demand as it can be arranged to involve a heavy excretion against an enormous concentration gradient of substances foreign to

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the kidney. For this reason we have found it worth while to examine the influence of the excretion of para-amino-hippuric acid (PAH) on the oxygen consumption of the human kidneys.

METHODS

The experiments were carried out on five human subjects, all women. Three were cases of neurosis without any signs of kidney damage, one suffered from a severe diabetes but had no kidney symptoms and one was a case of arterial hypertension with a slight albuminuria and isosthenuria but no casts or haematuria.

Arterial blood was taken through an indwelling needle inserted in a brachial artery during the entire experiment. Renal vein blood was taken through a Cournand catheter put into the right renal vein through the heart from the left brachial vein. The urine bladder was emptied by a catheter and after each period washed twice with saline $(2 \times 20$ ml). Inulin was given according to Alving and Miller (1940) in one single injection of 100 ml 10 per cent solution. In each experiment PAH was first injected intramuscularly according to Bucht (1949) in order to get a plasma concentration below the depression limit. Now the renal oxygen consumption was studied during two periods with light tubular work. After that we gave an intravenous injection of 50 ml 20 per cent PAH as its sodium salt. Inulin was analysed according to Josephson's and Godin's (1943) modification of the method of Corcoran and Page, PAH according to Smith, Finkelstein, Aliminosa, Crawford and Graber (1945) and oxygen according to Van Slyke and Neill (1924). The samples for oxygen analyses were taken under paraffin. The clearance periods were usually 15—17 minutes, occasionally as low as 11 or as high as 21 minutes.

The tubularly excreted PAH (T_{PAH}) was calculated by subtracting the filtered PAH (inulin clearance times arterial plasma PAH concentration) from totally excreted PAH. The renal blood flow was calculated from the arterio-venous differences of the plasma concentrations of PAH, the PAH in the urine and the hematocrit values, except in experiment 1 where it was calculated

from the arterio-venous inulin difference, as the PAH difference was too small to be reliable as a basis for calculation. The oxygen consumption was calculated from the arterio-venous difference in whole-blood oxygen concentration and the renal blood flow. In calculating the clearances and the tubular excretion we have taken no consideration to the delay-times. This may involve some errors when using short clearance periods as the delay-times according to Brun, Hilden, Raaschou (1949) may be as long as 5 to 15 minutes.

RESULTS

The results of the five experiments are briefly reported in Table I. In the first experiment the inulin clearance (glomerular filtration) decreased considerably in connection with the large intravenous PAH injection (indicated in the table by an arrow →), in the other experiments it rose without any considerable rise in the renal blood flow or the blood pressure. A maximal tubular PAH excretion of between 56 and 88 mg per minute was reached shortly after the large intravenous PAH injection but the output was high even during the following period. The fact that in some experiments we found the maximal tubular excretion to occur during the second period after the intravenous injection, not during the first one, may by explained by the delay-time which might be of some influence when these short periods are used. Josephson (1947) found similar results with diodrast.

COMMENTS

No appreciable increase of the renal oxygen consumption during heavy tubular excretory work was observed. In the fifth experiment the oxygen consumption was increased about 50 per cent during the first period after the intravenous injection only

Table I.

Period nr and time for intr. ven. PAH-inject.	Diuresis ml/min.	Inulin clear. ml/min.	PAH plasma conc. mg/100 ml.	A–V PAH-diff. mg/ml.	T_PAH mg/min.	Renal blood flow ml/min.	A–V O₂-diff. ml/100 ml.	O₂-consumpt. ml/min.	
1	4.6	119	2.3	0.9	13	1280	0.85	10.9	Woman 32 y
2	→ 2.8	116	2.7	0.7	11	980	1 25	12.3	b. s. a.
3	8.5	117	78	0.6	22	905	1.35	12.2	1.65 m²
4	3.8	63	44	3	88	622	1.60	10.0	neurosis.
1	7.7	118	0.9	0.5	3	1330	1.05	13.3	Woman 29 y
2	7.5	119	0.9	0.6	2	830	0.95	7.9	b. s. a.
3	→ 7.5	118	0.7	0.5	2	710	1.05	7.4	1.50 m²
4	11.5	105	42	24	22	526	1.20	6.3	neurosis.
5	12.9	85	61	9	9	984	1.35	13.2	
1	10.1	161	1.0	0.9	2	670	1.30	10.0	
2	7.1	131	0.8	0.7	4	1190	1.55	18.5	Woman 43 y
3	→ 6.0	139	0.6	0.5	5	1520	1.30	19.7	b. s. a.
4	9 5	179	47	22	85	1000	1.10	11.0	1.54 m²
5	7.3	153	34	19	81	1020	1.10	12.6	neurosis.
6	7.0	161	26	18	77	1030	1.20	12.3	
1	6.5	117	3.0	2.6	14	1065	1.45	15.5	Woman 20 y
2	2.1	117	2.3	1.8	9	1070	1.55	16.6	b. s. a.
3	→ 5.7	232	-	-	-	-	1.50	-	1.65 m²
4	4.6	120	58	25	84	1000	1.60	16.0	diabetes
5	5.5	218	24	17	64	1030	1.70	17.5	No kidney
6	3.3	174	17	15	50	880	1.55	13.7	symptoms.
1	1.9	74	4.2	3.8	7	400	1.75	7.0	Woman 52 y
2	2.2	95	3.7	3.2	9	580	1.65	9.6	b. s a.
3	→ 4.7	191	-	-	-	-	1.80	-	1.56 m²
4	2.0	126	32	28	13	695	1.90	13.2	arterial
5	3.6	118	17	13	56	670	1.60	10.7	hypertension

Renal oxygen consumption in heavy tubular excretion.

to go down to the original level already in the next period when the tubular excretion was maximal. In the second experiment the consumption even decreased, in the others it was very little influenced. As the arterio-venous oxygen differences are rather constant in each of the subjects the observed variations of the oxygen consumption are more or less parallel to variations in the renal blood flow. The lack of increased oxygen demand during heavy tubular work shows that the kidney gets the energy for this work too not by an increased metabolism but by an increase of its efficiency.

The relative constancy of the arterio-venous oxygen differences before and after the large PAH injection together with the absence of an augmentation of the renal blood flow make it probable that no Trueta (Oxford) shunt had been caused by the injections or at least none of measurable extent. On the contrary, these injections

seem to have reduced the total renal blood flow in the same way as diodrast injections sometimes do. We believe this to be due to a constriction of the afferent arterioles in some cases and the efferent arterioles in others as it is sometimes followed by an increase, in others by a decrease of the filtration fraction.

SUMMARY

By means of catheterization of a renal vein in man it was shown that heavy tubular excretion of para-amino-hippuric acid was not accompanied by an increased oxygen consumption of the kidneys.

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SUGAR REABSORPTION IN PREMATURES AND FULL TERM BABIES

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Premature babies display at birth a physiological immaturity of varying degree, which decreases with advancing age an which seems to be independent of the birth weight.

The investigations into the sugar reabsorption which will be reported in this paper comprises 14 premature babies aged from 1 to 103 days and, for comparison, 7 full term babies, aged from 9 to 50 days. A total of 32 series of examinations has been made in these 21 infants.

The experiments were arranged as follows: A permanent needle was inserted through the skin into a vein of the skull or of one of the extremities. Two containers, one containing 0.9 per cent saline solution, the other a 5 per cent solution of glucose, were connected with the needle by means of a three-way stop-cock. The rubber tubes leading from the containers to the three-way stop-cock are provided with drop counters and clamping screw to adjust the rate of infusion. The infusion of saline solution was started 1½' hours before the actual investigation in order to secure a sufficient output of urine. At the same time inulin was administered by hypodermic injection. During the experiment the blood sugar was determined at intervals of 10 minutes (Hagedorn-Norman Jensen method) and the plasma inulin was determined 3 or 4 times

by a micromethod elaborated by Vesterdal(3). A single clearance period generally comprised 10 minutes. After 1 or 2 clearance periods the three-way stop-cock was turned so as to replace the infusion of saline with glucose infusion; the latter was continued until sugar was present in the urine, when the infusion of saline solution was resumed. The bladder was evacuated by means of a catheter á demeure.

In this manner a total of 409 clearance periods was determined, all calculations having been corrected according to a surface of 1.73 m².

The filtered sugar was calculated as a multiple of inulin clearance and the blood-sugar, and the reabsorbed sugar as the difference between filtered and excreted sugar.

After a rise of the blood-sugar up to 300 mg per cent, the output of urine became very high. The highest diuresis ascertained by me was 25.2 cc per minute per 1.73 m² of surface in a premature. Owing to the risk of dehydration no experiments were made with a constantly increased blood-sugar level and, consequently, no actual attempts were made at determining the glucose Tm. No injurious consequences were observed during these experiments.
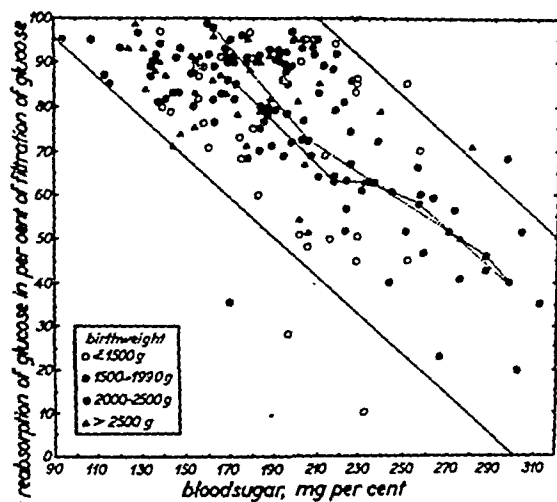
Fig. 1. Reabsorption of glucose in per cent of the filtered glucose in relation to the blood-sugar. The dotted line connects points originating from the same series of examinations.
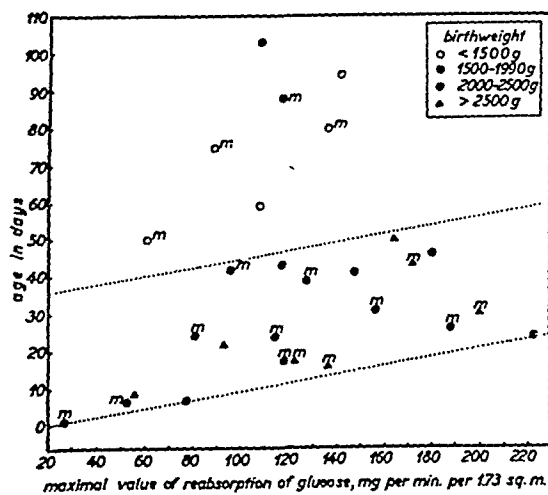
Fig. 2. Maximum reabsorption of glucose in relation to age at the examination. The points marked *m* presumably indicate the glucose Tm.

The reabsorption of glucose expressed in percentage of the filtered glucose during these experiments has been plotted in Fig. 1 in relation to the blood-sugar. The values

of the single clearance periods in the course of the sugar excretion have been marked. It is seen that the reabsorption of sugar decreases when the blood-sugar rises; this drecrease takes place within a certain range of variation and is independent of the birth weight.

When points originating from the same series of examinations with rising and falling blood-sugar are connected, lines are found (e. g. the dotted line in Fig. 1) which are approximately parallel. In repeated examinations of the same individual the position of the lines is almost identical.

A reabsorpion less than 80 per cent of the amount of filtered glucose is found in a great number of cases, which indicates that many of these values correspond to the glucose Tm (Goldring et al. (1)).

In Fig. 2 the maximum values of reabsorbed glucose in the individual series have been plotted in relation to the age of the infant. The values marked *m* were found in examinations where the amount of reabsorbed glucose is less than 80 per cent of filtered glucose, and are supposed to correspond to the glucose Tm.

From the rise of the lower points in Fig. 2 it may be supposed that the glucose Tm, corrected to the standard surface in the newborn, will attain the adult value at the age of 2 months at the earliest.

Two or three examinations in the same child at different ages show that the reabsorption of glucose rises with advancing age, but that children with a birth weight above 2000 gm show a more rapid rise than children with a birth weight under 1500 gm.

Similarly, it is found that the amount of reabsorbed glucose increases with increasing
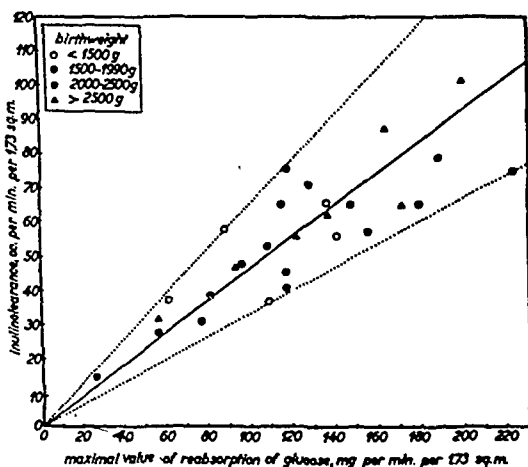
Fig. 3. Maximum reabsorption of glucose in relation to inulin clearance. The solid line represents mean values for all examinations, the dotted lines indicate the extreme values

weight during the experiments, the youngest babies in each weight-group always showing the lowest reabsorption of glucose per minute.

The ratio inulin clearance : glucose Tm has been found by Smith(2) to be 0.395 — with variations from 0.30 to 0.50 — in adult females. In the present material, which chiefly comprised girls, the ratio inulin clearance : maximum reabsorption of glucose was 0.47 with variations from 0.34 to 0.66 (Fig. 3). This is somewhat higher than the value found by Smith in adults, indicating that the reabsorption of glucose

both in prematures and in full term babies is more reduced than the filtration, the two functions keeping fairly well abreast with advancing age up to 103 days.

SUMMARY

After correction according to the standard surface the reabsorption of glucose at birth both in prematures and full term babies is less than in adults but shows a tendency to rise with advancing age and increasing weight, most slowly in premature babies weighing under 1500 gm at birth. It is supposed that the glucose Tm also rises with advancing age, but that values corresponding to the glucose Tm in adults cannot be found before the child is over 2 months old, and in premature babies weighing under 1500 gm at birth even considerably later. During the first two months of life the glucose reabsorption is found to be more reduced than the filtration, and this phenomenon is independent of the birth weight and of age.

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GLOMERULAR FILTRATION RATE AND MAXIMUM TUBULAR EXCRETORY CAPACITY IN DIABETIC NEPHROPATHY (SYNDROME OF KIMMELSTIEL AND WILSON)

By T. HILDEN

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In cases of diabetic nephropathy, Hogeman(6) studied inulin clearance and diodrast clearance and found a more pronounced decrease in inulin clearance than in diodrast clearance, consequently the filtration fraction showed low values. As no determination of maximum tubular excretory capacity was made, such determinations were taken up in this paper, the thiosulphate clearance being determined simultaneously. As will be seen in the present paper, the reduction of the filtration rate and the tubular function are equal. While these investigations were being made Corcoran, Taylor and Page(4) have published experiments showing the same results.

TECHNIQUE

The glomerular filtration rate has been determined be means of Na-thiosulphate, the tubular excretory capacity by means of diodrast.

After a primary injection the plasma concentration of these substances was kept constant by means of a constant intravenous infusion. Analyses of thiosulphate were made by the method of Brun(2), diodrast by the method of Bak, Brun and Raaschou(1). Urine collection was performed through catherization and at the end of each clearance period the bladder was washed out twice with 50 cc water plus 50 cc air.

PATIENT MATERIAL

In 1936 Kimmelstiel and Wilson(7) described the syndrome called intercapillary glomerulosclerosis and since that time many reports on this syndrome have appeared. It hase been stated (5, 8, 10) that the glomerular lesions described by Kimmelstiel and Wilson are seen at autopsy in a large percentage of diabetics. It seems likely, therefore, *that in diabetics such glomerular lesions are* present together with other renal diseases e. g. chronic glomerulonephritis, chronic pyelonephritis or malignant hypertension. This may explain why we occasionally see patients with symptoms suggesting the syndrome of Kimmelstiel and Wilson, but at the same time showing anamnestic and laboratory signs of other chronic renal diseases. Some patients, however, seem to belong to a special group; they are the *cases with diabetes of long duration, without* anamnestic signs of renal disease, but with marked proteinuria usually combined with decreased serum albumin concentration, and further with retinopathy and some degree of hypertension. Such cases may be diagnosed as diabetic nephropathy or as the syndrome of Kimmelstiel and Wilson, and it may be suspected that intercapillary glomerulosclerosis is the underlying cause. The patients studied in the present paper belong to this category. The data of the patients examined are shown in Table I.

Table I.

No.	Sex	Age	Duration of diabetes in years	Proteinuria in ‰	Urine sediment		Culture from urine	Concentration of serum albumin	Blood pressure	Eye ground
					red cells	white cells				
1	F	66	10	6	(+)	+	+ coli	2.72	240/130	III
2	F	47	24	2.2	÷	+	÷	3.15	100/100	? (cataract)
3	F	65	13	1.0	÷ ·	·∔·	÷	3.80	210/100	III
4	F	59	18	1.0	÷	(+)	÷	?	155/90	III
5	F	24	20	0.4	÷	(+)	÷	5.30	190/100	III
6	M	59	31	2.5	÷	(+)	÷	2.00	155/90	II
7	F	34	14	2.0	÷	+	÷	2.15	195/115	III

Clinical and laboratory data from 7 cases of diabetic nephropathy.

Table II.

No.	Number of clearance periods	Plasma concentration in mg/100 cc plasma		Thiosulphate clearance		Diodrast — Tm.		G. F. R./Tm.
		Thiosulphate	Diodrast	cc/min.	% of normals	mg/min.	% of normals	
1	3	49	30	16	14	5.2	12	3.02
2	2	35	26	19	16	11.9	27	1.60
3	4	50	41	49	42	18.1	41	2.70
4	2	41	38	60	51	27.8	63	2.15
5	3	31	19	75	63	38.9	88	1 93
6	3	47	22	76	61	26.6	53	2.86
7	3	32	26	89	75	32.3	73	2.75

Glomerular filtration rate and maximal tubular excretory capacity for diodrast in 7 cases of diabetic nephropathy.

RESULTS

The results are shown in Table II. In the columns in which the figure of the renal tests are expressed in percentage of normal values, these values are taken from the normal material investigated in our laboratory(3) and they are shown in Table III.

DISCUSSION

From the experimental results presented above it will be seen that the glomerular filtration rate and the maximal tubular excretory capacity of diodrast are depressed to the same extent.

As the patients with diabetic nephropathy often show edema, this retension of sodium and water may suggest a glomerular tubular

Table III.

	Mean normal value	
	Men	Women
Glomerular filtration rate	125	118
Diodrast Tm.	50.6	44.2
G F. R./Tm.	2.51	2.69

Mean values for glomerular filtration rate, diodrast Tm. and G. F. R./Tm. from the normal material of Brun, Hilden and Raaschou (3).

imbalance, the glomerular function being decreased more than the tubular function. Although a change in the diodrast excretion is not necessarily quantitatively parallel to

alterations in sodium reabsorption the present data speak against a marked functional imbalance.

Moreover it is a common clinical experience that diabetics with Kimmelstiel and Wilson's syndrome often show very little or no glucosuria despite of rather high blood sugar levels, pointing to a high threshold or sauration limit for glucose. This again may be explained as a result of a more pronounced depression of the filtration rate causing a low load to the comparatively better working tubular cells. The results from the diodrast-Tm determinations here presented speak against such a functional imbalance. Further Porsborg and Lundbæk(9) in diabetes of long duration have found glucose Tm to be decreased to the same extent as the filtration rate, in some patients even more depressed. Further investigations are needed if we shall be able to explain the high saturation limit of glucose in older diabetics.

As previously stated the diagnosis of Kimmelstiel and Wilson's syndrome is difficult, and as a rule it can only be made as a tentative one. If the renal function pattern could give us some aid in making the differential diagnosis, this should be greatly appreciated. Judging from the investigations of Hogeman(6) and Coronan, Taylor and Page(4) together with those of the present paper the renal functional pattern in cases of diabetic nephropathy seems to be a more or less, but an equally, decreased glomerular and tubular function, while the renal blood flow is not decreased to the same extent, the filtration fraction consequently being low.

This pattern resembles that of the chronic glomerulonephritis in the nephrotic stage, so it is of no help in distinguishing this syndrome from that of Kimmelstiel and Wilson.

On the other hand the pattern of diabetic nephropathy differs from that of malignant hypertension in respect to the filtration fraction, this being markedly increased in the last mentioned condition. Consequently in cases where blood pressure and retinopathy may suggest a malign hypertension a low filtration fraction speaks strongly against this diagnosis.

SUMMARY

In 7 cases of suggested diabetic nephropathy (syndrome of Kimmelstiel and Wilson) the glomerular filtration rate and the maximal tubular excretory capacity for diodrast (diodrast Tm) are found to be decreased to the same extent.

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ON THE EXCRETION OF SALICYLIC ACID IN MAN*
(A PRELIMINARY REPORT)

By M. BJØRNEBOE, S. DALGAARD-MIKKELSEN AND F. RAASCHOU

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The excretion of salicylic acid in man takes place almost exclusively in the urine where from 80 to 90 per cent of the quantity administered may be recovered in the form of compounds with intact salicylic radical. These compounds are: salicylate and the so-called conjugated salicylic acid compounds consisting of salicylurate, which is an equimolecular compound of salicylic acid and glycine, and salicylglucuronic acid compounds, the configuration of which has not been agreed upon.

The conjugated compounds cannot be demonstrated in the blood and, therefore, their synthesis must be referred to the excretory organ itself, the kidneys. In blood plasma, salicylate has been detected only after administration of salicylic acid; a very large proportion of the latter is connected with the plasma proteins, as is seen in Fig. 1, where the concentration of plasma salicylate has been plotted along the abscissa and the concentration in the ultrafiltrate along the ordinate. It appears from the curve that the percentage of freedom (F) increases with rising plasma concentrations.

When the clearance of the total quantity of salicylic acid compounds is determined at

different pH values in the urine, it appears — as shown in Fig. 2 where all the clearance periods of experiments on 6 different subjects have been plotted — that the clearance is very low in acid urine (5 to 10 ml/min.), begins to rise at pH 6, and at a pH value of 7 it rises considerably, so as to approach the value of the thiosulphate clearance. This relation between the pH of the urine and the clearance completely resembles the course of the bicarbonate excretion.

Mention may be made of the practical consequence of the low salicylic acid clearance in acid urine and the rising clearance in alkaline urine: clinical treatment with

Fig. 1.

THE FUNCTION OF THE URINARY TRACT AS ›DEAD SPACE‹ IN CLEARANCE EXPERIMENTS

(A PRELIMINARY REPORT)

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Renal clearance estimations have always presented a "dead space" problem, and the investigations reported here were carried out to clarify the function of the various parts of the urinary tract in this respect. The practical implication of the results obtained is that they offer methods to obtain a true clearance in spite of variations in the concentrations of the clearance substance in the urine, either due to variations in diuresis or the blood concentration.

The transport of urine from its formation as glomerular filtrate until delivery into the bladder is here supposed to take place as follows: As regards the type of fluid transport, three subdivisions should be considered: the tubular system, the renal pelvis and the ureter. In the tubular system it seems likely that in each section there is a complete mixing transversally since the small dimensions should permit a complete equalization of concentration differences; in addition, in the collecting tubules, the joining of the streams must cause a mechanical mixing. On the other hand no longitudinal mixing should take place in this subdivision. In the renal pelvis, composed of infundibulum and calyces, the whole pelvis content will be continuously and completely mixed by the muscular contractions of the walls. In the last subdivision, the ureter, the fluid does not flow, but is transported by peristalsis. In short, the tubules and the ureter will delay the urine excretion, without mixing urine originating from successive moments, and the pelvis will mix the urine continuously without reducing the maximal rate of urine transport. In the present experiments, the bladder will not contribute to the dead space because it has been completely emptied through a catheter. If our suppositions are correct, it should be easy to find the delay in the excretion of urine caused by the tubules and ureter. This time will be called "the minimum excretion time". One will merely have to inject a dye intravenously and note when it appears in the urine. As this "appearance time" also includes the circulation time of the dye from the vein to the renal artery and the time the urine spends passing through the catheter from the bladder, these last two intervals must be subtracted.

If the blood concentration varies, it is necessary to displace the period of urine collection "minimum excretion time" in relation to the curve of blood concentration.

The part played by the renal pelvis in the "dead space" problem is a more intricate one, due to the continuous mixing. The possibilities for an estimation of this volume will be considered next.

ON THE EXCRETION OF SALICYLIC ACID IN MAN[*]
(A PRELIMINARY REPORT)

By M. BJØRNEBOE, S. DALGAARD-MIKKELSEN and F. RAASCHOU

From Medical Department III, Kommunehospitalet, Copenhagen and the Department of Pharmacology, University of Copenhagen

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The conjugated compounds cannot be demonstrated in the blood and, therefore, their synthesis must be referred to the excretory organ itself, the kidneys. In blood plasma, salicylate has been detected only after administration of salicylic acid; a very large proportion of the latter is connected with the plasma proteins, as is seen in Fig. 1, where the concentration of plasma salicylate has been plotted along the abscissa and the concentration in the ultrafiltrate along the ordinate. It appears from the curve that the percentage of freedom (F) increases with rising plasma concentrations.

When the clearance of the total quantity of salicylic acid compounds is determined at different pH values in the urine, it appears — as shown in Fig. 2 where all the clearance periods of experiments on 6 different subjects have been plotted — that the clearance is very low in acid urine (5 to 10 ml/min.), begins to rise at pH 6, and at a pH value of 7 it rises considerably, so as to approach the value of the thiosulphate clearance. This relation between the pH of the urine and the clearance completely resembles the course of the bicarbonate excretion.

Mention may be made of the practical consequence of the low salicylic acid clearance in acid urine and the rising clearance in alkaline urine: clinical treatment with

Fig. 1.

287

Fig. 2.

Fig. 4.

Fig. 3.

When a differentiation of the salicylic aci compounds in the urine is undertaken an the distribution of the individual component is examined at different pH values, as show in Fig. 3, it appears that the linked com pounds, above all salicylurate, are predomi nant in acid urine; free salicylate constitute only about 15 per cent, but in the alkalin direction the proportion of free salicylate rises and at pH 7.5 it constitutes about 4C per cent of the salicylic acid compounds excreted.

We have attempted a closer analysis of the cause of this rise of the excretion of free salicylate. Fig. 4 shows an experiment in which the excretion of a highly acid urine was first achieved by means of administration of ammonium chloride, after which a gradual rise of pH was produced in the course of the experiment by means of infusion of a sodium bicarbonate solution, whilst a continuous infusion was used to produce a gradual rise of the concentration of salicylate in the blood. The excreted and the filtered quantities of salicylate have been plotted along the ordinate, and it is seen that the quantity excreted does not exceed that filtered until the pH is 7.5, which must be

salicylate-bicarbonate mixtures should be avoided, as this will promote the excretion of salicylate.

When the clearance of total salicylic acid, which comprises both the free salicylate and the conjugate compounds formed in the kidney, is determined at different plasma concentrations (from 15 to 30 mg per cent) and at a constant pH of the urine, no self-depression has been observed.

Fig. 5.

considered indicative of a reabsorption of salicylate in acid urine. Besides being due to the change of pH, the rise of the excreted and filtered quantities has also been produced by the rise of the concentration in the blood in the course of the experiment. If the pH of the urine is forced in a still more alkaline direction, a mode of excretion like that shown in Fig. 5 is achieved; it appears that at pH over 7.3 the excretion exceeds the filtration, i. e. we have to do with a tubular excretion.

As is known, Cooke, Barclay and Kenney put forward a few years ago the theory that there are substances which are both filtered, reabsorbed and excreted in the kidney ("the three-component system"). They mentioned urea and inorganic phosphate as examples. However, it has not been proved of any of these substances that they are excreted in the tubules. With regard to salicylic acid, this seems to be at least a three-component, or rather a four-component substance, as it

is filtered, reabsorbed and excreted in the form of free salicylate and is also excreted in the form of conjugated compounds after tubular synthesis.

In Fig. 5 it looks as if salicylate is reabsorbed in acid urine and excreted in alkaline urine and as if only filtration but neither resorption nor excretion take place at a certain pH value (7.25). It is possible that this is actually the case; however, we are inclined to believe that excretion and reabsorption take place within the entire pH range but that these processes tend to disguise one another, so that a predominant reabsorption takes place in the acid range, whilst the excretion prevails in the alkaline range. If it becomes possible to block up one of the tubular processes, we hope to find out what actually takes place.

If, finally, we were to consider why the kidney reabsorbs salicylate, perhaps even salicylate of tubular excretion, in the acid range of the urine, it would be reasonable to suppose that this is subservient to the acid-base regulation of the organism. When the supply of acid to the kidney exceeds the supply of base, the organism will require cations, which are then presumably resorbed again in the distal tubules together with the bicarbonate ion. It is possible that the salicylate ion may supplement the bicarbonate ion in the renewed resorption of cations. Conversely, when the supply of base to the kidney is preponderant, the excess of cations is excreted together with available anions, including salicylate.

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THE FUNCTION OF THE URINARY TRACT AS »DEAD SPACE« IN CLEARANCE EXPERIMENTS
(A PRELIMINARY REPORT)

By E. BOJESEN

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Renal clearance estimations have always presented a "dead space" problem, and the investigations reported here were carried out to clarify the function of the various parts of the urinary tract in this respect. The practical implication of the results obtained is that they offer methods to obtain a true clearance in spite of variations in the concentrations of the clearance substance in the urine, either due to variations in diuresis or the blood concentration.

The transport of urine from its formation as glomerular filtrate until delivery into the bladder is here supposed to take place as follows: As regards the type of fluid transport, three subdivisions should be considered: the tubular system, the renal pelvis and the ureter. In the tubular system it seems likely that in each section there is a complete mixing transversally since the small dimensions should permit a complete equalization of concentration differences; in addition, in the collecting tubules, the joining of the streams must cause a mechanical mixing. On the other hand no longitudinal mixing should take place in this subdivision. In the renal pelvis, composed of infundibulum and calyces, the whole pelvis content will be continuously and completely mixed by the muscular contractions of the walls. In the last subdivision, the ureter, the fluid does not flow, but is transported by peristalsis. In short, the tubules and the ureter will delay the urine excretion, without mixing urine originating from successive moments and the pelvis will mix the urine continuously without reducing the maximal rate of urine transport. In the present experiments, the bladder will not contribute to the dead space because it has been completely emptied through a catheter. If our suppositions are correct, it should be easy to find the delay in the excretion of urine caused by the tubules and ureter. This time will be called "the minimum excretion time". One will merely have to inject a dye intravenously and note when it appears in the urine. As this "appearance time" also includes the circulation time of the dye from the vein to the renal artery and the time the urine spends passing through the catheter from the bladder, these last two intervals must be subtracted.

If the blood concentration varies, it is necessary to displace the period of urine collection "minimum excretion time" in relation to the curve of blood concentration.

The part played by the renal pelvis in the "dead space" problem is a more intricate one, due to the continuous mixing. The possibilities for an estimation of this volume will be considered next.

If V is the volume in the urinary system, in which a complete mixing takes place ("the mixing volume"), and M the amount of clearance substance which has been delivered to the bladder during the experimental period t, at initial and final concentrations R_1 and R_2, then the following formula will give the true clearance:

* $$Cl_{true} = \frac{M + V(R_2 - R_1)}{t \cdot P_m}$$ (1)

Here P_m is the average plasma concentration corrected for minimal excretion time.

If two substances with identical clearances are used simultaneously with blood concentrations varying at different rates, the following formula can be used to estimate V, since all the other figures are measurable.

$$\frac{M + V(R_2 - R_1)}{t \cdot P_m} = \frac{m + V(r_2 - r_1)}{t \cdot p_m}$$ (2)

Using this formula, changes in filtration rate or diuresis could perhaps introduce errors in the stimation of V by washing out tubular contents. However, without entering into a detailed discussion of this question, it seems likely that the errors caused by changes in diuresis are insignificant. The material does not justify any conclusions regarding the influence of changing filtration rate.

How does the volume V, calculated from the formula (2) compare with the volume of the renal pelvis. If the picture of the fluid transport in the urinary system drawn in the introduction is correct, these two volu-

mes should be equal. To settle this question it became necessary, in addition to the indirect measurement of V using formula (2) immediately after, to measure the pelvic volume directly. Briefly the technique used was as follows:

1) *indirect method*: Exogenous creatinin and inulin were chosen as the two substances with identical clearance. Dogs of 20—23 kg body weight were used. Different diureses were produced by intravenous infusions of hypotonic NaCl solutions.

2) *direct method*: Immediately following an indirect estimation of V, ligatures previously placed around the renal vessels and the ureter were tightened, the kidney was removed, and the volume of fluid in the pelvis was estimated by injecting a known amount of an alien substance in the pelvis. After mixing, the final concentration was measured.

Comparison between V and the pelvic volume.

Three membutal anaesthetized dogs were used in these experiments. The results are shown in Table I and it is seen that the volume V does not differ by more than 0.5 ml from that of the pelvis.

The relation between V and the diuresis

The pelvic volume was further investigated by the indirect method alone in a series of experiments carried out on 6 non-anaesthetized dogs. It soon became apparent that the pelvic volume increased with increasing diuresis. This relation is shown in Fig. 1. All dates plotted represent periods in which widely different rates of change in blood concentrations for the two substances have been obtained.

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* It is readily seen that in cases of constant diuresis and blood concentrations, R_2 wll equal R_1 and the formula thus be reduced to the familiar clearance formula $M/t \times F_m$.

Table I.

	Left pelvis		Right pelvis	
	Indirect method	Direct method	Indirect method	Direct method
K	0.5 – 1.0 ml	0.5 ml	2.8 ml	3.0 ml
F	1.4 ml	1.5 ml	0.0 ml	0.5 ml
M			4.3 ml	3.8 ml

The relation between direct and indirect estimation of the volume of the renal pelvis in three dogs.
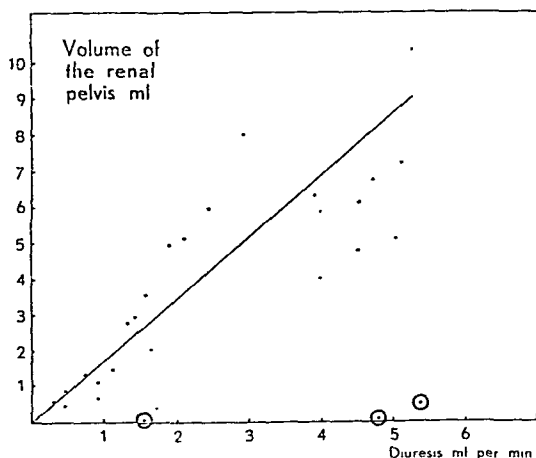
Fig. 1. The relation between the volume of the renal pelvis and the diuresis.

On the whole, the mixing volume, V, is seen to be roughly proportional to the diuresis. However three of the dates plotted — marked by circles — differ much from the majority. Since in all these cases the diuresis has been rapidly increasing (more than 2. per cent per min.) this might be the reason for the discrepancy. This assumption would seem to be supported by the fact that, among the other dates, those representing periods with rapidly increasing diuresis are preferably located below the curve. It does not seem unlikely that the pelvic volume actually is diminished in periods with increasing diuresis, as it is well known

that a stretch stimulus produces a contraction in unstriated muscles.

As the relation between V and diuresis is rather constant, when the diuresis is not increasing too rapidly, it should be possible to get an approximate estimate of V by merely measuring the diuresis, when working on dogs of size similar to that used in my experiments.

Practical implications

Below is a discussion of how the results reported can be utilized to obtain true clearance values in dogs in spite of changes in blood concentration of the clearance substance and in diuresis.

Constant diuresis and a regularly changing blood concentration: It is possible to calculate by which time interval (the so called "total delay time") one will have to displace the blood concentration curve in relation to the urine collecting period, in order to find the real clearance, in spite of increasing or decreasing blood concentrations. As will be seen later, this mathematical concept "total dalay time", has a meaning only if the diuresis is perfectly constant throughout the period, and the following formula is derived with the assumption of constant diuresis.

The concentration of the clearance substance in the pelvis V is given by the following equation:

$$V \cdot \frac{dR}{dt} = Cl \cdot p(t) - R(t) \cdot D$$

in words: the rate of increase of the amount of substance in the pelvis is the rate of intake minus the rate of output. The symbols are V pelvis volume, R(t) concentration of substance in pelvis at time t, D diuresis per min., Cl clearance for the substance, and p(t) the plasma concentration at time t. Mr. Poul H. Rasmussen has integrated this equation for a logarithmically decreasing blood concentration according to $p(t) = p(o) \cdot e^{-ht}$ to:

$$R(t) = \frac{p(o) \cdot Cl}{D - hV} e^{-ht} + I \cdot e^{-\frac{Dt}{V}} \qquad (4)$$

The invariable I is given by the concentration of substance in the pelvis at the beginning of the period. If the blood concentration has decreased at a constant rate in a certain time, I will be zero. In the case of a logarithmically changing blood concentration curve, Poul H. Rasmussen has developed a formula by means of which one can calculate the minimum time, that will have to elapse before I becomes zero. The amount of substance m delivered to the bladder during the time t becomes

$$m = D \cdot \int_{o}^{t} R(t) \cdot dt$$

$$= -\frac{(p_t - p_o) \cdot Cl \cdot D}{(D - hV) \cdot h} \qquad (4)$$

$$m = -\frac{(p_t - p_o) e^{-ht} \cdot Cl \cdot D}{D \cdot h}$$

Here only one new symbol is introduced. It represents the "total delay time", which is ... vis. From the one can find:

$$e^{h\tau} = 1 - h\frac{V}{D}$$

$$\tau = \frac{1}{h} \cdot \ln\left(1 - h\frac{V}{D}\right) \approx -\frac{V}{D}$$

If the blood curve falls linearly:

$$\tau = -\frac{V}{D}$$

according to an analogous deriv...

Therefore *the total delay time plus minimum excretion time.*

From the curve in Fig. 1 it i... find the volume of pelvis V... measuring the diuresis and he... total delay time. I have teste... for "total delay time" in the... from Fig. 1 showing pe... diuresis, and have found go... between this and a value ... namely the time interval ... place the urine collecting p... tion to the concentration c... get the same clearance ... them, determined by the ...

From the formula (4) ... one for linear blood cur... possible to find V ... employing the formula ... not necessary to measur... and R_1. The drawbac... for constant diuresis.

In short, for mor... determinations, the pr... justify the following ... constant diuresis and ...

blood concentrations the clearance should be corrected by means of total delay time $\frac{D}{V}+$ minimum excretion time. In periods with moderately varying diuresis, it is necessary to use the formula:

$$Cl_{true} = \frac{M + V(R_2 - R_1)}{t \cdot P_m}$$

using an estimation of V from D, and in addition a minimum excretion time correction.

Advice concerning the conventional clearance technique: It can be shown that the error introduced in clearance estimations due to the pelvic volume will vary parallel to the ratio $\frac{V}{D}$. According to Fig. 1 this ratio seems roughly constant independent of the diuresis. Thus to try to diminish the error caused by pelvic dead space by increasing the diuresis within the range investigated will be irrational.

Further experiments and details concerning the experimental technique will be published later on in Acta physiologica scandinavica.

SUMMARY

A hypothesis concerning the fluid transport in the urinary tract is presented. A formula is presented which, by taking the effect of the dead space into account, makes it possible to determine the true clearance. Determinations of the volume of the renal pelvis based on this formula and determinations by a direct method have given consistent results. Implications of the results are discussed.

THE PROTEIN CONTENT OF THE DIET AND THE FUNCTION OF THE KIDNEYS IN HUMAN BEINGS[1]

By A. LEVIN NIELSEN and H. O. BANG

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Since the results of Addis & Drury's research were published in 1923, it has been known that when human beings are given protein, the urea clearance (U. cl.) rises. The rise generally takes place one to three hours after the meal, and may amount to 40 per cent. Various amino—acids can produce a similar rise. This rise lasts only a few hours, so that there is no change in the clearance values on an empty stomach, no matter whether the amount of protein in the diet varies from 75—100 g or from 100—280 g in 24 hours. These figures refer to the U. cl. In 1948 White & Rolf reported, on the basis of two experiments, that the inulin clearance (I. cl.) rises slightly after a high protein meal, without the paraamino-hippuric acid clearance (PAH cl.) showing any change, and the results of one experiment showed that 220 g protein a day for eight days caused a rise in the I. cl. of 31 per cent and in PAH. cl. of 18 per cent.

In order to investigate these results further, we have determined the values — on an empty stomach — for the I. cl., U. cl., and diodrast clearance (Diod. cl.) in six normal women aged from 20 to 24 years. This was carried out with high diureses in at least three periods of about twenty minu-tes each (continuous intravenous infusion of inulin and diodrast) with catheterization and rinsing. The values were determined on the basis of normal diet, the nitrogen content of the urine being on an average 7.9 g in 24 hours. After a diet containing 225 g protein a day for 5 to 7 days, with an average of 18.2 g N in the urine for the last 2 days, the experiment was repeated.
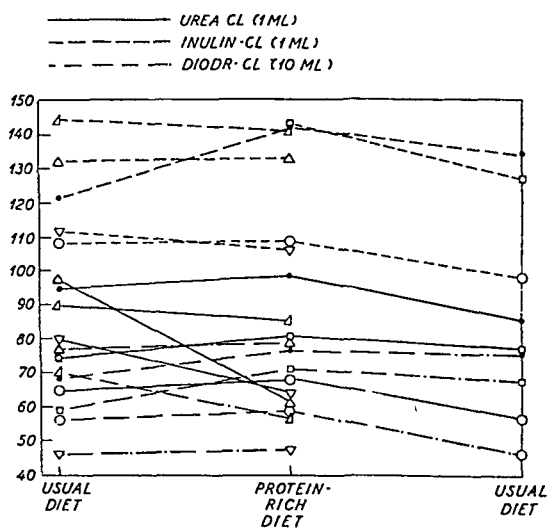
Fig. 1. Graph showing urea, inulin and diodrast clearances in 6 healthy persons. The values were determined on an empty stomach, either after a normal diet, or after a diet containing 225 g protein a day for 5 to 7 days. The ordinate gives the three clearances in ml/min., the diodrast clearance is, however, given in $\frac{ml/min.}{10}$.

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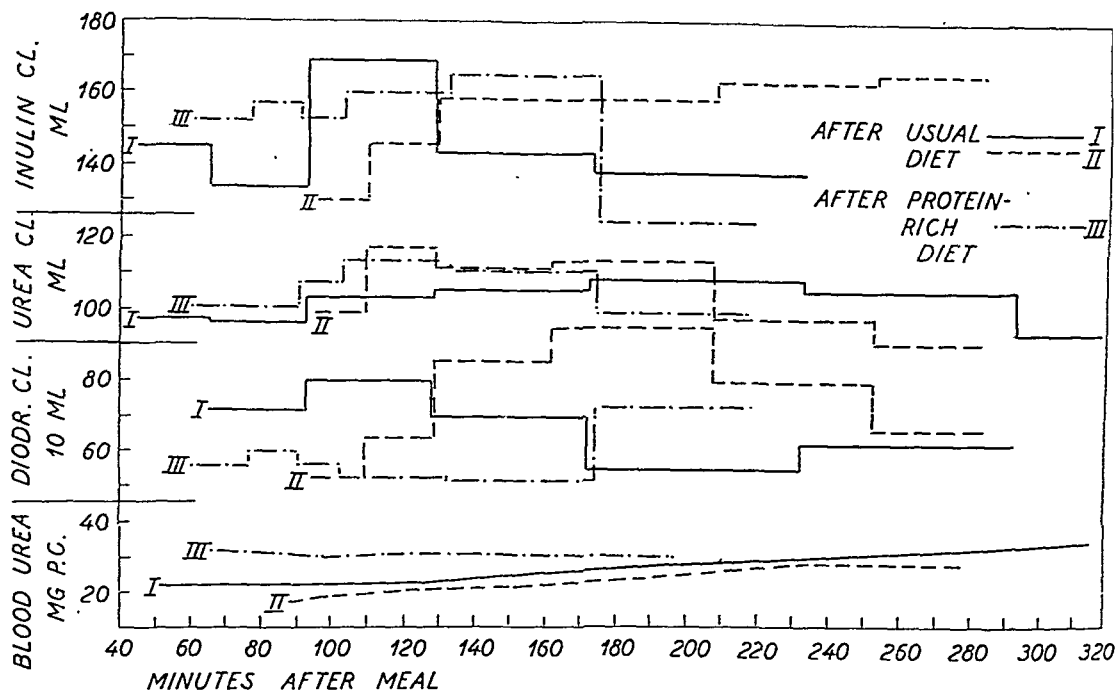
Fig. 2. Inulin, urea and diodrast clearances in anormal person, who for at least 5 to 7 days before the experiment had had a normal diet or a high protein diet. The abscissa shows the time in minutes between the high protein meal and the clearance. The blood urea during the experiments is indicated below.

In three of the cases a further control experiment was made after 7 days on a normal diet. The results are shown in Figure 1, which, however, has not been corrected for height and weight.

It will be seen from Figure 1 that the I. cl. is unchanged in 4 of the cases, and is higher than that of the control period in 2 of the cases. The U. cl. is unchanged in 4 cases and is less in 2 cases, in these 2 cases the I. cl. remaining unchanged. The Diod. cl. shows practically no change in 4 cases, a rise in one case, and a fall in one case.

Altogether it is possible to conclude from this that the clearance values on an empty stomach are not influenced in any particular

direction by a considerable increase in the protein content of the diet.

In another series of experiments, clearance values were determined directly after a meal consisting of 400 g of meat. These experiments were made on 3 of the above mentioned women, partly after a normal diet, and partly after a diet of 225 g protein a day for seven days. The following diagram shows a typical example of the results: (Fig. 2.)

Two experiments were made after a normal diet, and one after a high protein diet. In the first experiment on a normal diet, one subcutaneous and intravenous injection of inulin and diodrast were given, in the two other cases the method was continuous in-
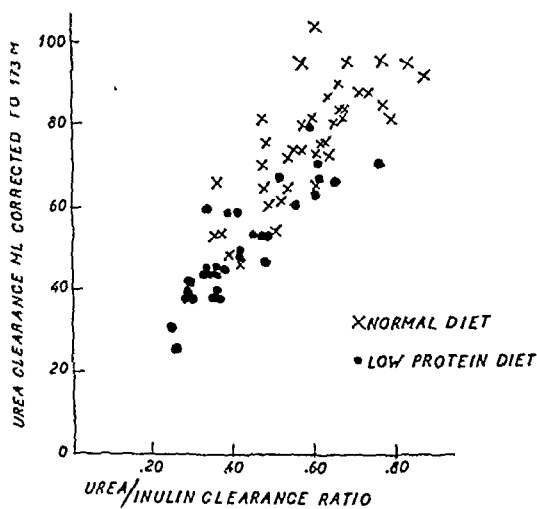
Fig. 3. Urea clearance/inulin clearance ratio plotted against urea clearance in 8 healthy persons, who during the period preceding the experiment had been on a normal diet or an low protein diet.

fusion intravenously. In all three experiments the U. cl. rose slightly about 100 minutes after the meal. It is worth noting that the U. cl. of these 3 cases was practically identical at the beginning of the experiments, in spite of the different diets given before. I. cl. likewise rose slightly, but more irregularly. The Diod. cl. showed highly varying results, which did not follow the results for the U. cl. and I. cl.

Blood urea rose on a normal diet, but remained practically unchanged after high protein diet.

The effect on the U. cl. was thus seen to be the most regular, regardless of the protein content of the diet, even when this varied from normal to above normal.

Cope (1933) and Goldring et al. (1934) showed that the U. cl. on a low protein diet fell about 30 per cent. In an earlier publication (Levin Nielsen & H. O. Bang(1948)),

we have confirmed this, and also shown that the fall in I. cl. was only 7 per cent, while the Diod. cl. was unchanged. These experiments were made on 8 healthy women. In 9 out of 11 cases with nephritis Bang (1949) achieved similar results, though in the 2 most severe cases, the U. cl. appeared to rise with a low protein diet, while at the same the uraemia improved.

Figure 3 shows a graph of the clearance results of the experiments on the 8 healthy women after a normal diet and after a low protein diet mentioned before. The urea clearance/inulin clearance ratio is plotted against the urea clearance. It will be seen from the graph that only when this ratio is low, is the U. cl. low, or, in other words, I. cl. is only very slightly affected by a low protein diet.

CONCLUSION

It has thus been possible to confirm that the U. cl. falls considerably on a low protein diet, and shows a temporary rise 1—2 hours after a high protein meal, the clearance determined on an empty stomach being unaffected by a high protein diet for some days. These alterations of U. cl. are rather selective, as I. cl. and particularly Diod. cl. are relatively unaffected by a rise or fall in the protein content of the diet.

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KIDNEY FUNCTION IN HEART FAILURE

By E. BLEGEN

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This paper is based upon experimental studies made by Dr. Knut Aas and myself. A complete report will be given later. Our purpose has been to find out what happens in a restricted part of the periphery i. e. the kidneys, in cardiac failure. We feel that our limited knowledge about the pathogenesis of cardiac failure may be due to the fact that studies have been directed mainly towards functions of the central organs, the heart, the large vessels, the lungs, and only to a much lesser degree towards the periphery. Still it is here that many changes of importance must occur. From this point of view studies of kidney function in cardiac failure may be of interest.

I would like to remark that when the kidneys play such an important part in clinical investigations as this symposium in itself shows, this is not only because 'of their vital importance but it is also due to the fact that the kidneys, for anatomical and physiological reasons, are very well adapted for research work.

It is known that the renal blood flow (RBF) in cardiac failure undergoes changes of another magnitude than the cardiac discharge. RBF usually shows a greater fall. This implies that in valvular lesions the blood flow from the heart not only decreases, but blood is diverted from some peripheral organs, i. e. the kidneys, to others.

A complete knowledge of all such peripheral changes in cardiac failure would be of great value. This diversion of blood from the kidneys has been held to be of importance through retension of sodium and consequent development of edema. Although the complete significance of these suggestions is as yet unsettled, it is nevertheless rational that the changed renal circulation in cardiac failure must have some consequence. It seems on the other hand unlikely that the kidneys are the only peripheral organs where such changes take place.

We have studied the clearances of sodium hippurate, inulin, sodium-thiosulphate, "endogenous creatinine" and urea in normals and in patients with valvular heart disease. The methods are well known, and will not be described here. We refer to an earlier paper (Aas and Blegen 1949).

Practically all indviduals have had a normal blood pressure Our patients have suffered from mitral stenosis, aortic insufficiency, aortic stenosis and some mixed lesions

We have mainly studied the effect of hypoxemia induced by inspiration of 9.5 per cent oxygen in nitrogen. The gas has passed through a wash bottle, a rubber bag and a mouth piece (nasal clip). Two resting clearance period of ten minutes each have usually been followed by two hypoxemia-

periods of five to ten minutes each. After that the patient has inspired 100 per cent oxygen for four to five minutes in the first half of a ten minute clearance period, and the experiment has been concluded with a ten minute resting period, i. e. altogether six clearance periods. Because of the lack of time we have to abstain from the discussen of a very important point: to what degree does the PAH method give reliable results as a measure of the renal blood flow in cardiac failure? In an earlier paper (Aas and Blegen 1949) we have held that the validity of the PAH method is sufficiently well founded by catheterizations of the renal vein, and the demonstration that the renal extraction of PAH in cardiac failure is practically normal. (A. J. Merrill et al. 1948, St. E. Bradley et al. 1948). It will be shown later that our results give considerable support to this view.

In regard to our own results all values from normal individuals lie within the normal limits. Deviations from the normal are therefore largely due to other causes than methodical errors. Once and awhile we have been disturbed by unexpected variations in RBF, caused mainly by nervous and psychic reactions in the experimental individual.

In patients with valvular heart lesions we have regularly found a reduction in the RBF. This was not unexpected as most of these patients were hospitalized because of their heart failure.

On the whole it has been found that the more pronounced the signs of cardiac failure, the greater has been the reduction in RBF. The glomerular filtration rate is reduced simultaneously, but usually not to the same extent, resulting in a more or less heightened filtration fraction (FF).

Detailed notes have been taken in regard to the presence of dyspnoea, cyanosis, liver congestion, dilatation of the veins on the neck or arms, pulmonary congestion (both by auscultation and by X-ray) and edema. Sometimes the pressure has been determined in the cubital vein. In some instances the circulation time has been determined by decholine. All this has been done with the idea of correlating RBF and glomerular filtration rate with signs of left or right + left cardiac failure.

Our results show that when right failure is evident, with enlarged liver or edema etc. the RBF is practically always reduced, the glomerular filtration rate is reduced to a lesser degree, the FF is increased. But it has been surprising that in patients without right failure but with only dyspnea on exertion, or at most slight orthopnea, we have also frequently observed a reduction in RBF. In eight out of thirteen cases of left or presponderant left failure the RBF was found as low as 600 ml or less full blood per minute per 1.73 m² surface. In fact only one of ten patients with right heart failure showed a more pronounced reduction in RBF than those without signs of right failure. This was a very unexpected result. This reduction in RBF can not be explained by venous congestion as we have no reason to assume a renal congestion in many of these instances.

One can of course postulate that more delicate methods for determination of venous pressure might give other results. But as far as we can see our results do not confirm with the theory that reduction in renal blood flow and glomerular filtration rate are of major importance for the development of cardiac edema. We have not come across similar findings in the literature. Our failure

Table I.

The renal blood flow and filtration rate in patients with cardiac failure

Subject	R. P. F.	Filtr.	R. B. F.	F. F.	Cardiac failure
Age	ml/min. per 1.73 sq. m			%	
51 man	474	120	948	25	Dyspnea on effort
52 man	356	91	598	26	— „ —
48 man	318	91	635	28	— „ —
					Congestive failure
55 man	401	159	728	40	Slight pred. left
70 woman	419	98	723	24	„ pred. left
66 woman	355	104	573	29	„ bilateral
43 woman	419	151	697	36	„ pred. left
78 woman	203	61	343	30	Severe bilateral
59 man	269	97	448	36	„ pred. right
57 man	313	109	512	35	„ pred. left
65 man	170	72	251	42	„ pred. left

to detect signs of right heart failure in many cases with significantly reduced renal blood flow ought to be followed up with more elaborate methods.

Just to give a visual impression some experiments are shown in Table I.

It is at present widely known that renal ischemia does not in itself cause hypertension. A clear demonstration of this can be found in cardiac patients. We do regularly find a significantly reduced RBF in patients without any increase in blood pressure. And this can certainly go on for years.

We have in our studies no determinations of the cardiac discharge. From other investigations e. g. by McMichaels is known that in valvular heart lesions the basal cardiac output rarely sinks below 2.5 liters per min. except just before death. This represents 50 per cent of the normal basal value. Long before this the renal blood flow will have fallen to perhaps 25 per cent of its basal value. RBF thus declines more rapidly than does the cardiac output. This is an example of a change in peripheral circulation in cardiac patients which can not be forecast by any examination of the heart or the cardiac discharge. It shows a deviation of blood from one organ to others. We must admit that there is still a great lack of knowledge about such peripheral changes in cardiac failure.

The effect of muscular exercise on RBF in normals and in patients with valvular heart lesions has been studied by us only in some few experiments reported in an earlier paper (Aas and Blegen 1949). Studies with about the same technique are known from other publications (Merrill and Cargill 1948, Mokotoff et al. 1948, Chapman et al. 1948). The renal reaction to muscular exertion seems to be the same in normals and in patients with valvular lesions although it seems probable they will develop more easily in the latter. The renal function in resting cardiac patients behaves as it does in the normal kidney during muscular excercise.

One could thus say that the resting renal function in cardiac failure acts as if charged by muscular work.

Table II.

The effect of exercise in normal and cardiac subjects

Subject	Period Min.		R. P. F.	Filtr.	R. B. F.	F. F.
			ml/min. per 1.73 sq. m			%
25 man	Rest	20	910	155	1540	17
Normal	Exercise	23	591	145	1000	25
23 woman	Rest	20	772	173	1265	22
Normal.	Exercise	20	326	89	535	30
64 woman	Rest	20	495	85	812	17
Aortic insufficiency	Exercise	18	270	71	443	26
48 man	Rest	20	600	134	1100	22
Pericarditis	Exercise	18	525	134	922	25

Some few examples of the effect of exercise in normal and cardiac subjects are seen in Table II.

The studies reported here concern mainly the effect of induced hypoxemia in 8 normals and 27 patients. Of these 25 had valvular heart lesions and 2 had auricular fibrillation only. If we now exclude the patients with aortic stenosis and consider all the rest as one group, the effect of induced hypoxemia in this group is a distinct increase in renal blood flow. The glomerular filtration rate usually does not follow, resulting in a slight depression of the filtration fraction, or at most an unchanged FF.

In 24 such experiments, 8 normals included, a significant increase in RBF was found 20 times. Four times we found only slight fluctuations in RBF. The average increase in RBF was 14 per cent of the resting values. Normals and cardiac patients reacted in the same manner on induced hypoxemia.

There is some uncertainty in regard to the interpretation of these findings. Homer Smith states (Kidney Lectures 1943) that increased renal blood flow (renal hyperemia) can be induced only by an increase in the metabolism, i. e. by the pyrogen method, provoked for instance by typhoid vaccine. It is likely that the metabolism increases somewhat during the periods with hypoxemia because of the increased ventilatory movements. The increased metabolism in cardiac failure is explained by the increased muscular exertion connected with the dyspnea (Duncan 1947). On the other hand we know that muscular exertion leads to a decrease in RBF and not to an increase. This is at least so when the exercise is performed by walking or pedalling. That the effect of respiratory muscular activity should be different is hard to understand. It seems improbable therefore that the increased renal blood flow during induced hypoxemia is caused by the increased respiratory activity itself, or by increased metabolism.

Not only normal individuals but also patients with valvular heart lesions and auricular fibrillation increase their RBF during induced hypoxemia. Frequently this signifies an increase in a previously existing

hypoxemia. This is a very interesting conclusion. It shows that the reduction in renal blood flow commonly found in valvular cardiac failure can not have been caused by hypoxemia. One may often hear the statement that in cardiac failure RBF must be reduced while the hypoxemia makes it necessary that blood is directed to more important organs than the kidneys.

Although the outcome is a reduction in renal blood flow, the primary cause of this does not seem to be hypoxemia itself.

Another interesting conclusion can be drawn: when increased hypoxemia in cardiac failure leads to increased PAH clearance, the extraction of PAH in the tubules can not have been significantly reduced beforehand.

That the extraction in cardiac failure by other methods has been shown to be practically normal has been mentioned above. It is nevertheless of great importance to note that our results agree, as the whole study is based on the conception that the PAH extraction by the tubules in our patients is practically normal.

We would now like to point out the reduction we found in RBF in patients with valvular lesions without signs of right failure. This seems to conform very well with the impression that hypoxemia can not be the determinative factor for the reduction in RBF. The true mechanism is unknown to us. Merrill et al. (1946) have found increased renin concentration in renal venous blood in chronic heart failure. However, this may probably be more the result than the cause of the reduction in RBF, as they are well aware. Other humoral factors may be responsible, or a reflex vasoconstriction may take place.

Our patients with aortic stenosis did not react with increased RBF on induced hypoxemia. In 10 such cases the RBF showed no significant increase, in 3 cases there was a definite decrease, in 4 cases the RBF was unaffected, and in 3 there were slight fluctuations. An average decrease of 3 per cent in RBF was found. This is quite contrary to the other group where a definite increase in RBF was found in 20 out of 24 experiments, and in none of the cases a definite decrease.

The difference appears still more convincing when one considers that the clinical diagnosis of aortic stenosis does not in itself imply that there is also a functional stenosis. In experimental animals the aortic ostium must be reduced to less than ¼ of its natural size before a change can be shown in the systolic discharge, the blood pressure or pulse form (Wiggers 1944) "Much smaller degrees of stenosis suffice, however, to produce loud systolic murmurs". As is known these murmurs play an important role for the diagnosis of aortic stenosis. All things taken into consideration the difference between the two groups in reaction to induced hypoxemia must be considered certain.

During induced hypoxemia the FF as a rule declined in normals and in patients with valvular lesions except aortic stenosis. In the latter, FF twice declined a litle, was unchanged or slightly heightened 8 times.

The following two tables will give an impression of some experiments with induced hypoxemia.

The question now arises. Why is it that patients with aortic stenosis do not react with increased RBF on induced hypoxemia

Table III.

The effect of hypoxemia

Subject	Period	Min.	R. P. F.	Filtr.	R. B. F.	F. F.
			ml/min. per 1.73 sq. m			%
43 man	Rest	10	582	119	1021	22
Normal	„	10	548	110	962	20
	9.5 % O₂	10	686	123	1202	18
	„	10	701	124	1231	18
	Rest	10	575	122	1009	21
	„	10	590	129	1033	22
64 man	Rest	10	381	98	694	25
	„	5	314	85	571	27
Vitium	9.5 % O₂	10	457	98	831	21
Cordis.	„	7	442	101	802	23
Fibrillation	Rest	10	494	92	898	17
	„	10	319	105	580	33

Table IV.

The effect of hypoxemia

Subject	Period	Min	R. P. F.	Filtr.	R. B. F.	F. F.
			ml/min. per 1.73 sq. m			%
72 woman	Rest	10	311	100	536	32
Aortic	„	10	358	95	617	26
Insufficiency	9.5 % O₂	8	519	119	896	23
	„	5	465	100	803	21
	Rest	10	305	66	526	21
	„	10	417	111	719	26
70 woman	Rest	10	433	123	746	28
Aortic	„	10	390	108	673	28
Stenosis	9.5 % O₂	5	390	116	673	30
	„	5	377	117	650	31
	Rest	10	328	101	566	31
	„	10	326	111	562	34

as both normals and patients with other valvular lesions do. We can not find in our material that the explanation lies in a difference in age.

We can here only point out that there are hemodynamic differences between the two groups i. e. in regard to the pulse pressure, the arterial pressure curves. These are, in aortic stenosis, characterized by a slower rise and a flatter form than in any other valvular lesion. In the radial artery this is felt as a pulsus parvus et tardus. It follows that in functional aortic stenosis the pulsatile movements of the organs must have a smaller amplitude than in normals or in other valvular lesions. Erik Ogden (1948) has discussed the possibility that the pulsatile movements of the organs, i. e. the kidneys, may play a role for the vascular tone and the blood flow. It may thus be that

smaller pulsatile movements of the kidneys in aortic stenosis affect their ability to react with increased blood flow. Now, if the same should take place in the brain of these patients, with reduced ability to increase blood flow when necessary, one might find an explanation of the attacks of unconsiousness and the sudden death which, to a certain degre, characterizes aortic stenosis. These things have never been satisfactorily explained.

Inspiration of 100 per cent oxygen in some instances seems to have had a peculiar effect on the renal blood flow. As expected RBF usually returns to the starting point when hypoxemia is discontinued. But once and awhile a greater fall in RBF is seen during inspiration of 100 per cent oxygen. This happens not only when RBF has been increased beforehand, but also in some instances where there was no increase.

If we count only those instances where RBF showed a subnormal value in the 100 per cent oxygen period and a higher value in the final resting period, this happened 9 times in 35 experiments. It is probably of importance that in all these experiments the individuals had been subjected to hypoxemia before the treatment with 100 per cent oxygen started.

In this connection observations from aviation medicine may be of interest. Pilots may experience a veritable black out when 100 per cent oxygen is put on at high altitudes. Or they may get visual disturbances or muscular spasms or other symptoms. The condition is usually transient, may only last some seconds. This effect has been called the "paradoxal effect of giving oxygen"

(Latham F. 1948). We have not systematically examined for such symptoms and signs, but lately we have seen one person who complained of dizzines and fainting sensation when 100 per cent oxygen was put on. The FF's in these instances were unaffected. It seems that administration of 100 per cent oxygen after a period of hypoxemia may lead to vascular spasm in the kidneys.

In conclusion it may be said that our simple experiments afford examples of special, defined, peripheral changes in circulation in patients with cardiac failure. These changes must be based on special qualities in the peripheral organ. By directing attention towards the periphery it may be possible to learn something more about the pathogenesis of cardiac failure.

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GLOMERULAR FILTRATION RATE AND MAXIMAL TUBULAR EXCRETORY CAPACITY IN CONGESTIVE HEART FAILURE

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During the recent years our viewpoints on the pathogenesis of congestive heart failure have been changing to a great extent and an increasing interest has been paid to the rôle of the kidneys in the development of cardiac edema. Several authors have been able to demonstrate that in congestive heart failure the renal plasma flow and the glomerular filtration rate are reduced (1, 3, 7, 8, 9). According to our present hypothesis for the excretion of NaCl through the kidneys (10, 11) a reduced filtration rate may cause sodium retention if the tubular function is not reduced proportionally. To date no results of tubular function tests in congestive heart failure have been published.

In the present paper determinations of the maximal tubular excretory capacity for diodrast in patients with congestive heart failure will be presented. As will be seen this tubular function is found to be depressed to the same extent as is the glomerular filtration rate.

TECHNIQUE

The glomerular filtration rate has been determined by means of Na-thiosulphate, the tubular excretory capacity by means of diodrast. After a primary injection the plasma concentration of these substances was kept constant by means of a constant intravenous infusion. Analyses of thiosulphate were done by the method of Brun (4),

diodrast by the method of Bak, Brun and Raaschou (5). Urine was obtained through catherization and at the end of each clearance period the bladder was washed out twice with 50 cc water plus 50 cc air.

The patients were cases of congestive heart failure. In none of the patients were there any anamnestic or laboratory signs of a primary renal disease; the experiments were performed 1—2 days after admission during which time the patients were confined to bed without being submitted to any other treatment.

RESULTS

The results are shown in Table I.

In the columns in wich figures of the renal tests are expressed in per centage of normal values, these values are taken from the normal material investigated in our laboratory (6) and they are shown in Table II.

DISCUSSION

As in earlier publications a more or less pronounced reduction of the glomerular filtration rate is found. Further it will be seen that also the maximal tubular excretory capacity for diodrast (Diodrast Tm) is depressed. The depression runs parallel to the depression of the filtration rate, although in 4 cases the reduction of Tm i less than that of filtration rate.

It might be expected that as the arterial venous oxygen difference in the normal kid-

Table I.

No.	Age	Sex	Diagnosis	Number of clearance periods	Plasma concentration in mg/100 cm Plasma		Thiosulphate clearance		Diodrast—Tm		G.F.R./Tm
					Thiosulphate	Diodrast	cc/min.	% of normals	mg/min.	% of normals	
1	56	F	Arterio-sclerotic heart disease	2	16	20	59	50	24.4	55	2.42
2	69	M		3	25	26	60	48	22.8	45	2.63
3	67	F		4	26	25	67	57	26.9	61	2.49
4	57	M		2	20	29	69	55	25.3	50	2.73
5	51	M	Luetic heart disease	4	27	26	90	72	39.1	78	2.30
6	55	M		3	20	23	104	83	49.5	98	2.10

Glomerular filtration rate and diodrast Tm in six cases of congestive heart failure.

Table II.

	Mean normal value	
	Men	·Women
Glomerular filtration rate	125	118
Diodrast Tm	50.6	44.2
G. F. R./Tm	2.51	2.69

Mean values for glomerular filtration rate, diodrast Tm and G. F. R./Tm from the normal material of Brun, Hilden and Raaschou (6).

ney is so small, a certain reduction in the renal blood flow should not nesessarily produce a decrease in the cellular functions of the renal tubuli. However, the present investigation shows that in congestive failure the tubular function as regards excretion of diodrast is far from normal.

In this connection it must be pointed out that Bradley and Bradley (2) in their experiments, in which they aim to produce increased renal venous pressure by means of abdominal compression, found an equal reduction of the renal blood flow, the filtration rate, and the Tm for PAH and glucose.

Judging from the present investigation a considerable glomerular-tubular imbalance in congestive heart failure seems rather unlikely; but the question is whether this may give us any clue as to the mechanism of the sodium retention in these patients. Here it must be stated that the diodrast Tm of course only affords information about a single function of the renal tubulus, and that the tubular reabsorption of sodium might possibly be influenced to a different extent than the excretory capacity. Furthermore we know, that as the normal excretion of sodium is so small in comparison with the filterd and reabsorbed amount of sodium, even a very small imbalance between the filtration and reabsorption will be able to cause considerable changes in the sodium excretion. By means of the function tests available such small disturbances will be hard to detect.

It is the author's opinion that much more has to be learned about the mechanism of sodium excretion in normals before we are able to interpret correctly the results of kidney function tests in congestive heart failure.

SUMMARY

In 6 cases of congestive heart failure the glomerular filtration rate and the maximal tubular excretory capacity of diodrast (diodrast Tm) are found to be decreased to the same extend.

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SOME OBSERVATIONS ON THE TRUETA RENAL VASCULAR SHUNT

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The new theory of renal circulation, which Trueta and his co-workers present in their monograph of 1947, is summarized by these authors as follows:

"The blood reaching the kidney has two potential routes through that organ and, according to circumstances, it may pass almost exclusively by one or other of these routes, or in varying proportions through each of them."

The medullary route continues through the juxtamedullary glomeruli, the efferent vessels of these glomeruli, and their derivative vasa recta, to the interlobular veins. This route implies a shunting away of the blood from the cortex, and the shunt was brought into operation by the stimulation of the sciatic nerve or the nervous plexus surrounding the renal artery, by the action of various drugs, staphylococcus toxin, etc.

In 1949 Goodwin, Sloan and Scott published an extensive study in rabbits, dogs, cats and monkeys of the Trueta renal vascular shunt. They considered their results to be "a demonstration of a neurovascular control of the distribution of renal blood under the rather extreme and unphysiological conditions of these experiments". By way of clearance determinations in cats and rabbits, Black and Saunders (1949) found such alterations in PAH and inulin clearance as might correspond to the "Oxford shunt'. These authors also observed a renal vein blood pressure in cats amounting to 70 cm of saline solution, implying "large communications between renal artery and vein."

Obviously, these new conceptions of the renal circulation give rise to many fresh suggestions and open up wide fields for future research. It was the author's intention to take up some problems in renal physiology in the light of recent results, but first of all, I deemed it necessary to work out a reliable method of producing the "Oxford" shunt, a task which was not so simple as might be expected. This paper is a report of a preliminary investigation concerning methods of demonstrating anatomically cortical ischemias under various conditions.

Before adopting in the hospital the abdominal aortography originated by dos Santos, the author started an experimental investigation on the action of various X-ray contrast solutions upon the renal circulation of cats, rabbits and dogs. The purpose of that investigation was inter alia to find out whether highly concentrated and irritating contrast media injected into the aorta could possibly produce a shunt through vascular reflexes. It may be stated here briefly that severe circulatory damage in the kidneys of these animals may be produced by such injections, although not of a nature that could be *clearly* interpreted as a shunt, even though the pictures sometimes might lead to such an interpretation. Since these experiments have no immediate bearing upon the present theme, they will not be described in detail here, but will be published elsewhere.

In some of them one kidney artery was clamped with a serrefine for about one minute, and it was necessary to control the effect of a temporary ischemia on the kidney circulation.

Following such temporary obstruction of the renal artery, the renal function is known to be greatly reduced. Verney and Vogt (1943) found that occlusion of the artery to one single remaining kidney for periods ranging between 2 sec. and 600 sec. produced contemporary suppression of urine flow, and in some animals (dogs) long lasting suppression of urine was observed following short

periods, (from 5 to 120 sec.) of arterial occlusion. Experimenting on dogs, Selkurt (1946) compared renal blood flow, calculated from the renal plasma clearance of PAH and hematocrit volume, with whole renal blood flow, measured simultaneously by a direct method. Following a twenty minute period of complete renal ischemia, he found a reduction in the volume of direct blood flow, but a still greater percentile reduction in the clearances of PAH acid and creatinine. He concluded that the disparity between blood flow and renal clearances may be a reduction in glomerular filtration pressure, created by the arteriolar vasoconstriction which follows the period of ischemia. Similar changes were found during hemorrhagic shock.

Studying the effect of shock on the kidney in dogs van Slyke and his co-workers (1944) found that the effect of clamping the renal artery for three hours produced analogous effects on the kidney function as a reduction of the renal blood flow (estimated by hippurate) from shock due to hemorrhage or hammering of the thigh muscles. A total renal ischemia of 2—3 hours duration caused reversible damage to nephrons. After removal of the clamp, the blood flow, at first, was only partially restored, and the clearances of excretory substances remained relatively lower than the blood flow, although restoration generally took place in a few days.

An analoguous depression of the renal function was demonstrated by Corcoran, Taylor & Page (1943) during the onset of shock due to partially occluding limb tourniquets in dogs. According to these authors, the depression was due to increased renal vascular resistance, essentially of humoral origin, although nervous stimulation was considered partly responsible. They also hinted at the possibility of arteriovenous shunting.

Westerborn (1937) studied the renal function by means of urography in rabbits after varying periods of total occlusion of the renal artery. He found that a restoration of the anatomical structure and the function of the kidney could be expected only if the ischemia had not lasted more than 1⅓ hours. The beautiful x-ray pictures in his work are very interesting, showing diffuse cortical calcifications as a result of a temporary ischemia of a certain duration. It is reasonable to interpret these calcifications as dependant on the remaining impairment of the cortical circulation in contrast to the better medullary blood supply.

From the above survey it is seen that shock due to hemorrhage, occluding limb tourniquets, or crush injuries, may cause a depression of the renal function which in many respects is similar to the sequelae of temporary renal ischemia.

The author, therefore, started an investigation on the distribution of blood within the kidneys after occlusion of the renal artery, with special regard to the possibility of producing an "Oxford" shunt in this way. This method has the advantage that it makes possible a comparison between the experimental kidney and a control kidney in the same animal, without seriously affecting the blood pressure or the general circulation of the animal.

TECHNIQUE

Rabbits ranging in weight from 2.4 to 5 kgm were anaesthetized with intravenous nembutal or urethan with supplementary ether if necessary. From a laparatomy incision a serrefine was applied to the renal artery on the left side in 8 experiments on the right side in one (No. 4) and on both sides in one (No. 9). In two animals the clamp also included the renal vein (No. 2, and right side of No. 9). Apart from the dissection necessary to free the artery and vein, no other surgical procedure was performed. The kidneys were left in situ and the intestines touched as gently as possible, except in rabbit No. 4, where the fat and connective tissue surrounding both kidneys were removed before application of the clamp. In experiments 3 to 10, the laparatomy incision was closed during the time of renal ischemia, and the animals were placed lying on one side in a well heated basket. In experiments 1 to 5 a cannula was then inserted into the abdominal aorta from below with the point of the cannula above the origin of the renal arteries. At the time desired 15 to 20 ml of a solution consisting of 1 part of Higgins India ink and 2

Table I.

The distribution of blood within the kidney following total occlusion of the renal artery.

Rabbit No.	Anaesthesia	Time of occlusion of renal artery	Time of nephrectomy resp. inj. of ink after release of clamp	Staining	Result	Control kidney
1	Nembutal ÷ ether	4½ min	Immediately	India ink.	Normal distribution	Normal
2	Nembutal ether	25 min.	2 min.	India ink.	Normal	Normal
3	Nembutal ether	1 hr. 35 m	Immediately	India ink. Benzidin	Peripheral cortical ischemia, medullary vessels well filled	Normal
4	Nembutal ether	40 min.	1 min.	India ink.	No definite diff. from control	Normal
5	Urethan ether	1 hr. 45 m	Immediately	India ink.	No definite diff.	Normal
6	Urethan ether	1 hr. 40 m	10 min.	Benzidin	Pronounced cortical ischemia, medullary vessels well filled	(Solitary kidney)
7	Urethan ether	1 hr. 45 m	10 min.	Benzidin	Pronounced cortical ischemia, medullary wessels well filled	Normal
8	Urethan ether	2 hr. 40 m	50 min.	Benzidin	Pronounaed cortical ischemia, medullary vessels well filled	Normal
9	Urethan	1 hr. 30 m	1 hr. 45 m	Benzidin	Normal?	Both sides clamped
10	Urethan	1 hr. 20 m	3 hours	Benzidin	Cortical ischemia, medullary vessels well filled	Normal
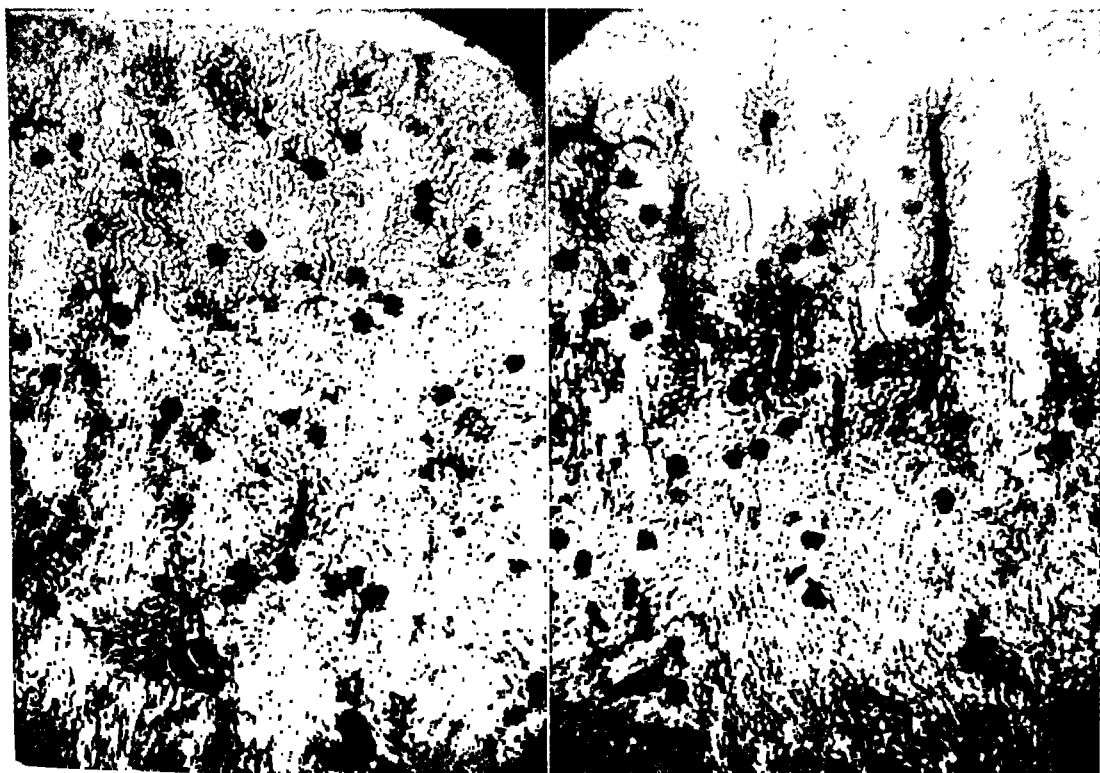
Right, control kidney.   Left, clamped kidney.
Fig. 1. Rabbit 3. India ink and benzidin.

parts of distilled water or normal saline were injected by a hand syringe. The whole injection was made in a few seconds. The injection pressure was not measured. In experiments 1 and 2 the mesenteric artery was ligated during this injection in order to avoid a disappearence of the ink into this vessel. Immediately after the injection the kidneys were quickly excised, after ligation of the artery and vein, and subsequently paraffin or freezing sections, varying in thickness from 50 to 150 μ, were prepared.

Since, however, the injection of India ink may involve a certain source of error, such as rupture and artificial dilatation of blood vessels, and circulatory changes due to altered pressure in the vascular area to be examined, the angioarchitecture of many specimens was studied by means of selective blood corpuscle staining with

benzidine our ortho-tolidin, as indicated by Sjöstrand. The advantages of this method are also beautifully demonstrated by Lindgren among others. Even after intravital injections, a supplementary staining with this method gives excellent results. In experiments 6 to 10 in this series the sections were stained in this way, and no other surgical procedure was carried out than the laparatomy and the clamping of the renal vessels.

RESULTS

A survey of these experiments is given in Table I.

Fig. 1 shows the sections from rabbit 3. A serrefine was placed on the left renal artery for 1 hr. 35 min.; immediately after release of the clamp, an injection of India

Right, control kidney. Left, clamped kidney.

Fig. 2. Rabbit nr. 7. Benzidin..

ink was made. The control kidney was at once stained black on the surface, whereas the left kidney showed only a few spotted black areas on the surface, although the ink was seen flowing in the renal vein. On section, the filling with India ink in the peripheral parts of the cortex is defective in comparison with the control side.

Fig. 2 illustrates the findings in rabbit 7, where the left kidney artery was clamped for 1 hr. 45 min. 10 minutes after release of the serrefine the kidneys were excised without any intravital injection or other experimental procedure, and the sections were stained with benzidine. In the experimental kidney the cortex shows a pronounced ischemia; only an occasional interlobular artery is seen, the glomeruli and the cortical network is very poorly stained. On the other hand, a series of "juxtamedullary" glomeruli and the vessels in the medulla appear amply stained. These changes were obtained in 5 of the 10 cases. In 7 cases the occlusion had lasted more than one hour, and a cortical ischemia was demonstrated five times. In rabbit 10 the ischemia was still demonstrable three hours after the release of the serrefine.

The results obtained after occlusion of one renal artery seemed to me very similar to pictures published in the monograph by

Table II.
Staphylococcus toxin.

Rabbit No.	Anaesthesia	Dose ml/kg	Survival time	Staining	Result
1	.	0.3	10 min.	Benzidin	No. definite diff. from normal
2	.	0.1	15 min.	Benzidin	Cortical ischemia, medullary vessels well filled
3	.	0.05	20 min.	Benzidin	D : o
4	.	0.03	8 hours	Benzidin	D : o
5	Nembutal + ether	0 2	3 min.	India ink. in agonem	India ink. unequally distributed in both kidneys with defective areas in cortex and medulla
6	Ether	0.04	15 min. later. in art. carotis	India ink.	No. definite diff. from normal
7	.	0.03	< 8 hours	Benzidin Orthotolidin	Cortex anaemic but not totally ischemic, medullary vessels well filled
8	.	0.03	< 8 hours	Benzidin Orthotolidin	D : o

Trueta and his co-workers. It was of interest to ascertain whether I could produce the same pictures by a closer imitation of the methods used in that monograph, namely with staphylococcus toxin, occluding limb tourniquets and faradic stimulation.

STAPHYLOCOCCUS TOXIN

Since staphylococcus toxin is known as a strong nephro-toxic agent, also used by Trueta and co-workers to produce cortical ischemia, the author performed a few such experiments in rabbits. Among those who have been working with this toxin, Ahlström (1936) should be mentioned in his monograph on glomerulonephritis. Ahlström concludes that staphylococcus toxin is a vaso-toxic agent affecting especially the kidney vessels, sometimes leading to cortical necrosis.

TECHNIQUE

The toxin used in the present experiments was prepared at the State Bacteriologic Institute, Stockholm, and kindly given to me by Dr. A. Lithander, who elaborated the method for its preparation. Details concerning this toxin may be found in Lithander's paper (see bibliography). The toxin was injected into an ear vein. In rabbits 15 and 16 a laparotomy was performed under anaesthesia and, after injection of the toxin, the usual ink solution was injected into aorta, resp. the carotid artery. In the other experiments in this series no anaesthesia was used and no surgical procedure performed. The kidneys were removed post mortem, except in rabbits No. 15 and 16. A survey of these experiments is given in Table 2.
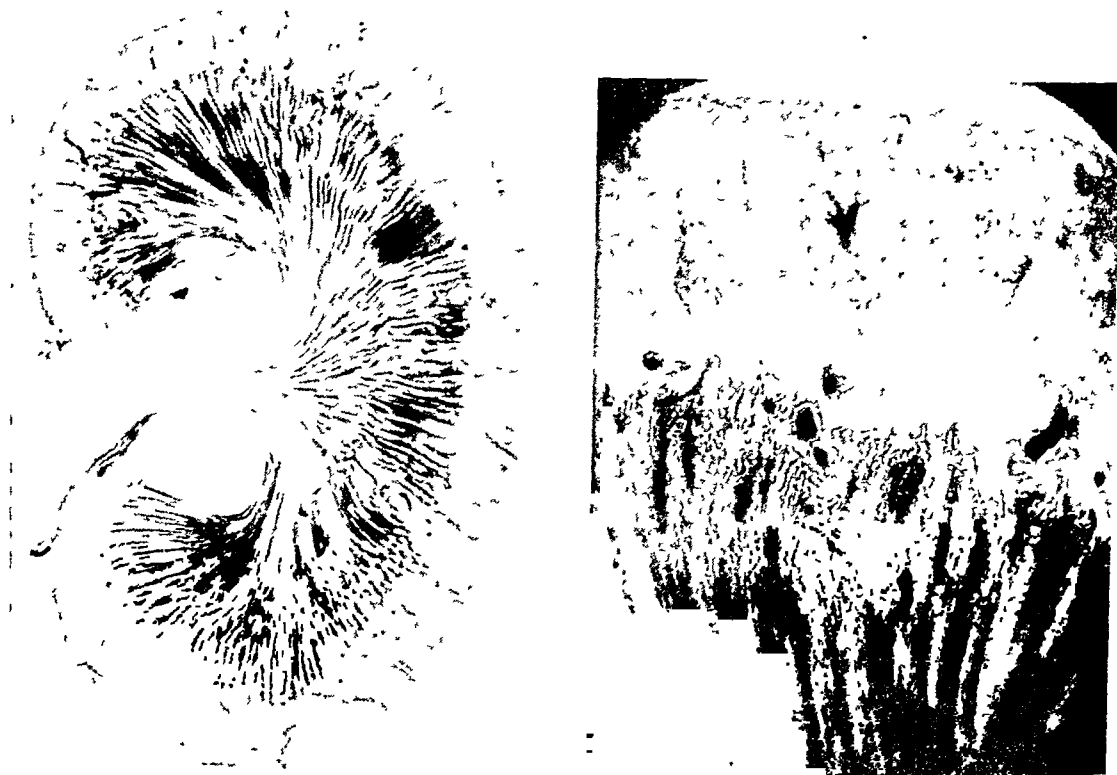
Fig 3   Rabbit 14   Benzidin

A cortical ischemia was demonstrated in five out of eight cases, although more or less pronounced. It appears that the dose of the toxin should be chosen so that the animal survives at least for a few hours, in order to produce a cortical ischemia with any degree of certainty (as Ahlstrom has pointed out).

Fig. 3 illustrates the findings in rabbit 14 The cortex with its glomeruli is practically empty of red blood corpuscles, but the medullary vessels are well filled. In this case even the juxtamedullary glomeruli are very poorly stained   This finding is not quite unique. On the contrary, the author has got the impression from these studies,

that the juxtamedullary glomeruli do not always play a predominant role. By means of steremicroscopy, whole series of medullary vessels can often be seen emerging directly from an arcuate vessel.

In rabbit 16 the ink was injected at a time, when the animal apparently was entering a state of shock, although the blood pressure was not measured. The ink staining appeared in the liver and abdominal wall in two to three seconds after the beginning of the ink injection, but no stain was seen on the surface of the kidneys, although it was clearly seen flowing in the renal veins. In the sections from these kidneys the ink staining is very poor both in

the cortex and the medulla. Blood corpuscle staining, however, shows that the cortex is comparatively anaemic, whereas the medullary vessels are more amply filled. Since, however, the quantitative estimation is difficult, the result in the table is designated as "no definite difference from normal". From the macroscopical finding during the experiment, it appears without doubt that the renal circulation is more affected than the circulation of the liver for instance, and that the ink solution passed the kidneys without appearing in the peripheral parts of the cortex.

Tourniquet experiments and faradic stimulation of certain nerves.

The author performed only a few experiments of this kind. In these the technique described in the monograph by Trueta et al. was followed as closely as possible.

In 4 rabbits a tourniquet was applied on the left thigh for five hours under nembutal anaesthesia. Within half an hour after release of the tourniquet, a laparotomy was performed, and the usual injection of India ink was done into the aorta. In three of these animals, where the blood pressure remained at a value of about 90 mm Hg, no definite changes in the renal blood distribution could be demonstrated. In one rabbit a beautiful cortical ischemia, with a comparatively rich blood supply in the juxtamedullary glomeruli and medullary vessels, was obtained in the left kidney (tourniquet side), whereas the blood supply of the right kidney appeared sparse in the cortex as well as the medulla, without the particular arrangement of the blood corpuscles that was seen on the left side. However, immediately before the injection of India ink, this animal unintentionally lost a large quantity of blood from the carotid artery, where the cannula for registration of the blood pressure was pulled out just before the injection of the ink. Thus, the injection was made during a state of hemorrhagic shock.

Faradic stimulation of the splanchnic nerve in two cats, of the tissue surrounding the renal artery in two rabbits ,and of the sciatic nerve in one rabbit, gave no definite divergence from the control kidneys. This failure may be due to my having used too low frequencies for the stimulation — at most 80/sec. For it is pointed out by Goodwin et al. that higher frequencies should be used.

Since the author's experiments of this kind are very few and the technique, therefore, not standardized, the results cannot be considered of great importance. It should also be emphazised, that minor divergences from the normal pictures are difficult to interpret, and it is safer for the beginner to induce more pronounced ischemias. I have not continued these experiments further, since I was able to induce the cortical ischemia in a more reliable way by the above-mentioned methods.

Finally, one experiment in a rabbit should be mentioned here, where *x-ray pictures* indicating an "Oxford" shunt were obtained.

Under urethan anaesthesia, a 50 per cent solution of diodrast was injected into the abdominal aorta and a set of x-ray pictures made. About 40 minutes later, the animal lost a large quantity of blood through the aortic cannula by mishap and became seriously affected. For the purpose in question — the examination of the action of diodrast upon the renal circulation — the experiment therefore was regarded as a
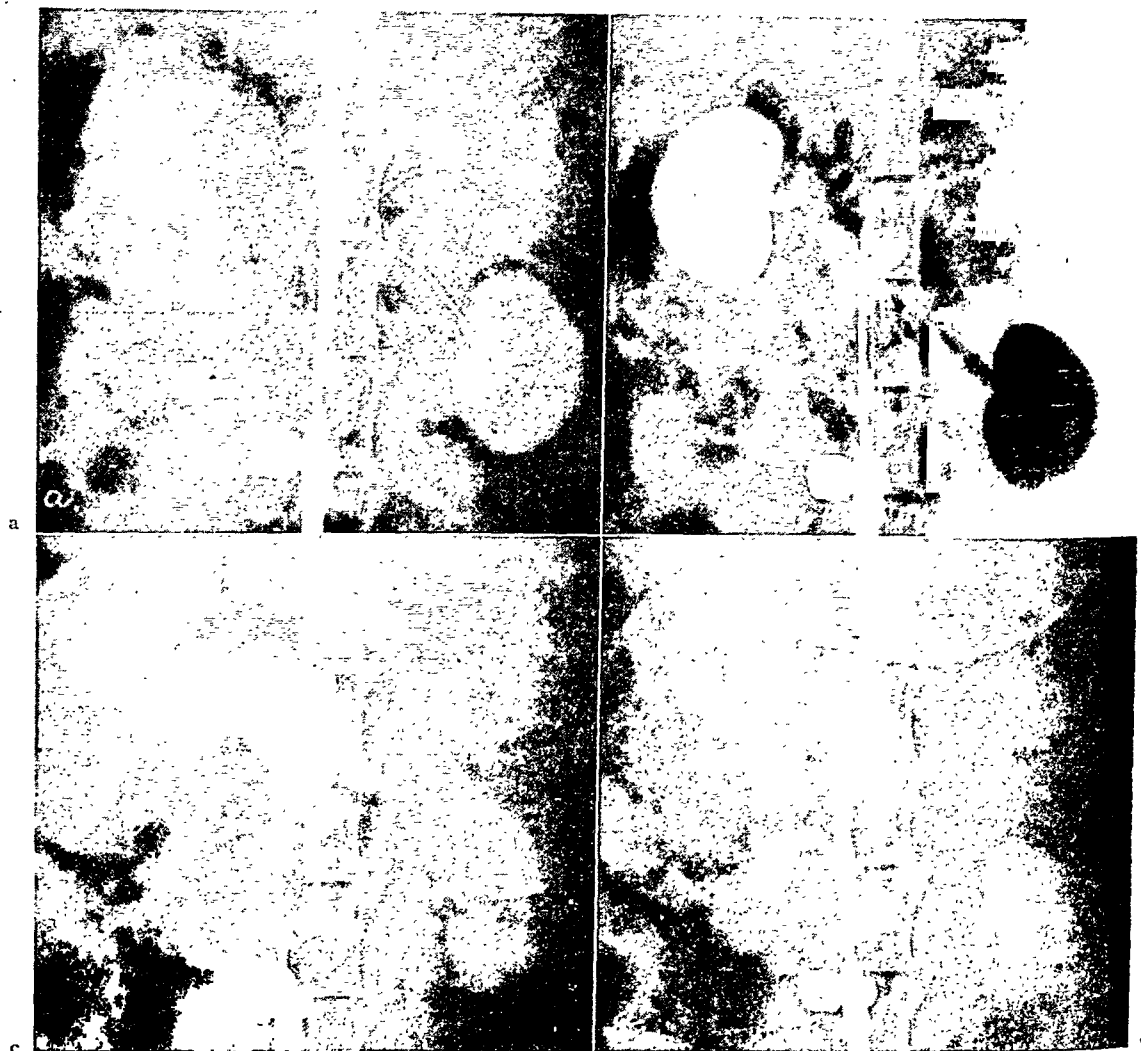
Fig. 4. Angiographs of a rabbit. a and b normal, arterial and venous stage resp. c and d 40 minutes later, when the animal was in a state of shock. Early and late arterial resp. The time interval between a and b was 3.2 sec., between c and d 3.3 sec.

failure. In spite of this, another injection of the same quantity of diodrast was made. The result is shown in Fig. 4.

In the first series, the density of the cortex contrasts clearly with the sparse opacity of the medulla. In the last series — immediately after the bleeding — the density of the cortex is very weak, whereas the central vessels are more clearly visualized. (Part of the shadow in the last two pictures is caused by the excretion of diodrast from the previous injection; a control picture immediately before the last injection, however, shows that this source of error is un-

Fig. 5. Boy, 5 days old. Benzidin.

important). The animal died half an hour later. Sections from the kidneys stained with benzidin show a certain but not total cortical anaemia. (From Palmlöv & Larsson). The result is interpreted as depending more upon the shock than on the immediate action of diodrast. Since operative trauma, chilling of the abdominal viscera, etc., per se may cause severe circulatory damage in the rabbit, we have not used the above method experimentally to demonstrate the shunting mechanism.

CLINICAL APPLICATIONS

It is natural that the work of Trueta and his collaborators should arouse the interest of the clinician and observations begin to appear in the literature in support of the shunting mechanism. Thus, Solymoss described a case of eclamptic anuria, where necropsy showed complete ischemia of the renal cortex and extreme hyperemia of the medulla, interpreted as a diversion of the blood flow.

Two such kidneys were kindly shown to me by Dr. G. Vejlens, The Pathological Institute, Sahlgrenska Hospital.

A section from one of them is seen in Fig. 5. The patient was a boy, who died from fever and attacks of cyanosis on the fifth day after he was born. The sections look like the ones obtained after staphylococcus toxin or renal artery occlusion: cortical ischemia and ample filling of the medullary vessels.

Exactly the same picture was seen in the kidneys from a man aged 27, who was found dead after a railway accident. He had pro-

Fig. 6. Normal aortogram of a woman aged 48 Early (left) and late (right) arterial phase
(From Palmlov & Larsson)

bably received a violent blow towards the trunk. As far as is known, he had previously been healthy. The diaphragm was torn and the aorta and vena cava were torn off with extensive bleeding into the abdominal and pleural cavities.

The last-mentioned case shows that these pictures must be interpreted with great caution. It is known that death occurred within a few hours but probably he was alive for only a few minutes after the accident. A sudden fall of the blood pressure may account for the peripheral ischemia, and it is not necessary to presuppose a redistribution of the blood flow in this case.

Clinically, in this hospital, *abdominal aortography* has been performed in some 50 cases in a variety of urological conditions. In successful cases, the difference between the contrast-filled cortex and the medulla is clearly seen, the cortex showing greater density. This is illustrated in Fig 6, which is the normal picture of the renal circulation in a woman, aged 48 In human beings,

this difference is not constant, owing to technical reasons, but we have seen no pictures indicating a diversion of the blood flow from cortex to medulla. However, this method has not been employed in patients in such poor condition that a high grade shunting could be expected. (From Palmlov & Larsson: Acta Radiologica in print.)

DISCUSSION

In the above experiments, different methods of producing renal cortical ischemia coincident with ample blood filling of the medullary vessels have been demonstrated On the other hand, it is difficult to answer the question, whether these pictures correspond to a shunting mechanism in the "Oxford" sense. The most reliable method was clamping of the renal artery or injection of staphylococcus toxin. It is reasonable to suppose that the renal blood flow under these rather extreme conditions was considerably reduced After clamping of the renal artery, this was the case in Selkurts experiments,

at least for an hour, following 20 minutes clamping. Whether the renal blood flow was still decreased after a three hours' interval in rabbit 10 in the present series is impossible to judge. That the cortical ischemia in this case was not caused by prolonged urethan anaesthesia is seen by comparison with the control kidney of the same animal. It is also probable that in my toxin experiments the renal blood flow was seriously decreased due to shock.

One great difficulty which presents itself in the interpretation of these pictures, is the quantitative estimation of the blood content in the medullary vessels. For, if a diversion of the blood from cortex to medulla takes place in kidneys whose total blood flow is normal, this will of course imply an increased flow through the medullary vessels. The author has not hitherto attempted to calculate the medullary blood content quantitatively, although such calculations would be possible statistically in big series, as demonstrated by Sjöstrand.

At present, it is therefore difficult to feel quite satisfied that the results in this investigation really imply a true diversion of the blood flow, although *they may well be explained on this basis.* The pictures, however, might be explained by a peripheral vasoconstriction as pointed out by Corcoran, Taylor & Page, Selkurt et al., in similar experiments.

It should also be emphasized that in estimating the distribution of blood in the vascular system, the state of the animal and the method of excising the organs in question may greatly influence the results, as pointed out by Sjöstrand (1935) et al. In the com-

ments to several experiments above, this is also stressed.

Now, since a diversion of the renal blood flow, or a cortical ischemia, can be more or less complete and since, above all, minor deviations from the normal findings are difficult to interpret, the author has thought it important to find methods of inducing a most reliable and most complete cortical ischemia as a basis for further studies, before an evaluation of more hypothetic conditions is attempted.

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Discussion

A. Bergstrand, Stockholm: Trueta et al. state that the "shunting" of the blood stream from the cortex to the medulla of the kidney, which they have described, may be brought about in a number of ways which seem to imply a stimulation of the sympathetic nervous system, and that the best result is procured by electrical stimulation of the renal stem. They do not state in more detail where the stimulus was applied, and it is not therefore known if it takes effect through the sympathetic nerve stems which lead to the kidney or acts upon the renal artery directly.

I have therefore isolated these nerves in the left renal stem of rabbits and stimulated them with a current from an inductor with a tension of 1 volt. Then India ink was injected into the abdominal aorta under a pressure which exceeded the systolic blood pressure in the carotid artery by about 10 mm. In the control animals the cortices of both kidneys were filled entirely with India ink.

The experiment covered 40 animals. Of these only one showed the distribution of the injection material in the cortex and medulla of the left kidney described by Trueta et al. and interpreted as a "shunting"

of the blood stream. The right kidney appeared normal.

In 20 animals no difference was observed between the two kidneys. The surface of the stimulated kidney retained its colour and the injection material filled the cortex completely.

In three cases the stimulated kidney had become decidedly pale and neither the cortex nor the medulla were filled with the injection material.

In the other cases it was observed that larger or smaller sections of the surface of the stimulated kidney became pale only to regain gradually their normal color, in the course of about five minutes. On section of the kidney a deficient filling of the cortex was found corresponding to its pale areas. This deficient filling extended to varying depths of the cortex and did not correspond in any definite way to an increased filling of the medulla and the juxta-medullary glomeruli.

Thus by using the technique just described I have only been able to evoke a picture corresponding to that described by Trueta et al. in one case in forty, and even this cannot be considered quite definite as no simultaneous red "streamline" was observed in the kidney veins. The investigation seems to show that stimulation of the nerve stems of the renal stem can evoke a contraction of different parts of the arterial tree with varying location, and varying degree of anaemia both in the cortex and medulla of the kidney.

THE RENAL EXTRACTION OF PARA-AMINO-HIPPURIC ACID AND OXYGEN IN MAN DURING POSTURAL CHANGES OF THE CIRCULATION

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Trueta and co-workers (1947) demonstrated that following manipulations of the large vessels and certain nerves in rabbits, there was an increased circulation through the renal marrow while the renal cortical circulation decreased. They and various authors tried to establish a parallel between this phenomenon and changes in the renal dynamics in various human diseases, above all hypertensive cardiovascular disease. So far only one group has tried to demonstrate this division of the renal circulation in living human beings. Reubi and Schroeder (1949) studied the effect of adrenalin and histamine on the renal extraction of para-amino-hippuric acid (PAH) and oxygen respectively. According to Trueta et al. these drugs caused a shunting of blood from the cortical to the medullary circulation. Reubi and Schroeder were not able to demonstrate any changes induced by the drugs.

According to Trueta et al. the medullary vessels are large channels that by pass the tubuli and empty the blood directly into the renal veins. The blood flowing through these vessels can thus not be completely cleared from the PAH, as this clearance to a large extent is a tubular function. Provided that the arterial concentration of PAH

is kept below the depression limit the concentration of this acid in the renal vein blood is thus a sign of the amount of blood passing through the medullary pathways. Of course some of the PAH may come from blood flowing through the renal capsule or through other vessels not in immediate contact with tubular cells. The extraction of PAH by the kidney is thus more or less proportional to the perfusion of the cortex if this cortex is capable to extract all the PAH passing through its blood capillaries. The higher the extraction, the smaller the blood flow through the medullary pathways, the lower the extraction, the larger the blood flow through extracortical channels. A second possibility to demonstrate a medullary shunt lies in the changes of the oxygen utilization by the kidney. The blood flowing through the large medullary vessels does not deliver oxygen to the tissues in contrast to what can be presumed of the blood flowing through the capillaries. A medullary shunting of blood trerefore would diminish the renal arteriovenous oxygen difference. This evidence was used by Trueta et al. in their animal experiments.

The technique of renal venous catheterization as developed by Cournand and Ranges (1942), Warren et al. (1944), Bradley et al. (1947) offers the possibility to obtain

renal venous blood in man and thus makes it possible to demonstrate an eventual induced medullary deviation of the renal circulation and also to a certain extent to calculate its magnitude.

The present work aims to study the effect of tilting on the renal dynamics and the extraction of PAH and oxygen. It has earlier been shown (Brun, Knudsen, Raaschou 1945) that tilting to the upright position diminishes the renal blood flow determined by the usual clearance technique. Tilting also influences the circulation in a way that resembles shock. Trueta and coworkers obtained their most striking pictures of renal shunting in experimental shock in rabbits. The diminished PAH-clearance in man during postural changes may thus be due to a similar mechanism.

MATERIAL AND METHODS

The study was performed in 4 subjects: Case A. L. was a 32 year old female, referred to the hospital for erythema nodosum and without signs of renal disease. Case A. W. was a 26 year old female with a slight chronic glomerulonephritis without blood pressure elevation and with a daily protein excretion of about 1 gm. She was 16 weeks pregnant when studied. Case K. B. S. was a 42 year old male with chronic glomerulonephritis and blood pressure elevation and a daily protein excretion of 1—2 gm. Case B. J. was a 22 year old female without signs of disease. She was 16 weeks pregnant when studied.

All the subjects were studied in the postabsorptive state. Before the procedure they had to drink at least one liter plain water. The right

renal vein was catheterized with the usual technique. An indwelling needle was placed in the right brachial artery. Six grams of creatinine were given by mouth 1 hour before the start of the experiment. The PAH was administered in a single intramuscular injection (Bucht 1949). Inulin was given in a single intravenous injection (Alving and Miller 1940). The first clearance period was started about 15 min. after the administraton of inulin and about 30 min. after the injection of PAH. Renal venous and arterial blood samples were taken at the beginning and end of each period. The urinary bladder was washed out twice with distilled water. The clearance periods usually lasted 15—20 min. The arterial blood pressure was recorded with the Tybjerg-Hansen-Warburg electrical condensor manometer.

After two clearance periods in the recumbent position the subject was tilted to 30° or 45° from the horisontal. If possible the clearance was determined during 2 or 3 periods in this position. In two of the subjects, however, subjective symptoms in the form of nausea, vomiting, vertigo or fainting prevented determination during more than one clearance period in the tilted position. When subjective symptoms of such severity appeared blood samples were immediately withdrawn and the patient tilted back to the recumbent position after which the bladder was immediately emptied. The other two patients did not experience such symptoms until the end of the third period. The clearance was subsequently determined during at least one period with the patient horisontal.

The procedure was always ended by administration of an amount of blood comparable to that which had been withdrawn during the procedure.

On all renal venous blood samples and every other arterial sample the oxygen content was determined on the Van Slyke apparatus. The oxygen capacity was determined on at least three blood samples.

PAH was analyzed according to Smith, Finkelstein, Aliminosa, Crawford and Graber (1945), inulin according to Josephson's and Godin's (1943) modification of the method of Corcoran and Page and creatinine according to Folin but with photoelectric colorimetry.

The clearance values were calculated in the usual manner, the renal plasma flow from the differences in PAH content in arterial and renal venous blood. The oxygen consumption was calculated as the renal arterio-venous oxygen difference times the renal blood flow calculated from the plasma flow and the hematocrit. The "medullary plasma flow" was calculated as the difference between the renal plasma flow and the PAH-clearance. Provided that the erythrocytes do not take up anything of the clearance substance any plasma clearance and especially the PAH-clearance must be identical with the total renal plasma flow if the *arteriovenous difference in plasma PAH concentration* is used instead of the arterial concentration alone. As a working hypothesis the PAH clearance calculated from the *arterial PAH-concentration alone* can be taken as that amount of plasma which passes the cortical way and which is extracted by the tubular cells. This interpretation, however, implies that the plasma passing the cortical way is completely cleared so that the blood leaving the parenchyma does not contain any PAH at all. We have no reason to believe that such a complete clearance takes place and on that account the calculation of the "medullary" flow is only hypothetical. A shunting of blood from the cortical circulation to a medullary pathway must, however, be reflected by changes in the hypothetical "medullary plasma flow" if it is calculated in this way. For that reason the "medullary plasma flow" is nevertheless of great interest in this investigation.

RESULTS

Fig. 1 shows the changes in PAH extraction during the four experiments. The results obtained when the subjects were recumbent are represented by drawn out lines, those from the tilting periods by dotted lines. There were no remarkable changes in the extraction during the periods of upright position in comparison to the values before or after tilting. In case A. L. (Table 1), though, the extraction decreased from 95 to 88 per cent. The changes in PAH clearances and renal plasma flow in the four subjects are also shown. In all cases except one (A. W.) there was a marked decrease in renal plasma flow and PAH clearance during the upright posture. Due to unaltered extraction the decrease in renal plasma flow paralleled the decrease in PAH clearance. Thus the calculated hypothetical "medullary flow" was unaltered during the whole procedure. Case A.W. had very high values for PAH clearance and renal plasma flow. She was studied in the 16th week of pregnancy, which may explain these values and perhaps also the difference in reaction to tilting. Table I gives all the calculated values of interest in the four experiments. Case A. L. with normal kidneys reacted to the circulatory changes with a decreased oxygen consumption, though both the blood flow and filtration decreased during the upright position.

DISCUSSION

An appreciable shunting of blood from the cortical to the medullary circulatory systems of the type described by Trueta et al. should be possible to demonstrate with the present procedure. In three of the

Case A.L

ml/min

Plasma flow and PAH clearance

%

The extraction of PAH by the right kidney.

cc/l

A-V O₂-diff.

10 30 50 70 90 110 min

Case B.I

ml/min

Plasma flow and PAH clearance.

%

The extraction of PAH by the right kidney.

cc/l

A-V O₂-diff.

10 30 50 70 90 110 min.

Case A.W.

ml/min

Plasma flow and PAH clearance.

%

The extraction of PAH by the right kidney.

cc/l

A-V O₂-diff.

10 30 50 70 90 110 min

Case K B S

ml/min

Plasma flow and PAH clearance

%

The extraction of PAH by the right kidney

cc/l

A-V O₂-diff.

10 30 50 70 90 110 min.

Table I.

Subject	Position	Inulin clear ml/min.	PAH clear ml/min	Renal Plasma flow ml/min.	Oxygen Consumpt ml/min.	PAH extract. %	„Medullary plasma flow" ml/min.	Arterial Mean Blood Press. mm Hg	Diures. ml/min.
A.L	Horisont.	141	588	620	25	96	32	94	9
	,	150	825	872	27	95	47	93	8
	30°	144	820	863	18	95	43	107	12
	30°	109	545	573	10	88	28	116	6
	45°	77	235	262	10	90	27	104	3
	Horisont.	152	766	790	34	97	24	98	2
B.J.	Horisont.	123	960	972	8	97	12	100	9
	,	128	1110	1130	12	98	20	98	14
	30°	100	634	642	6	98	8	80	9
	Horisont.	46	560	565	8	98	15	102	2
	,	146	1310	1320	26	98	10	-	2
	,	106	1280	1303	24	98	23	100	3
K.B.S.	Horisont.	89	296	353	4	85	57	144	17
	,	79	275	325	6	85	50	142	18
	30°	64	222	254	7	89	32	130	7
	45°	57	185	209	7	87	24	132	2
	Horisont.	74	237	273	8	85	36	127	3
	,	61	264	285	8	85	21	124	3
	,	73	222	259	7	85	37	132	4
A. W.	Horisont.	178	1050	160	14	90	160	-	2
	,	192	815	121	9	79	121	-	3
	45°	133	938	172	10	89	172	-	1
	Horisont.	76	880	85	9	93	85	-	0 5

experiments the total renal plasma flow was decreased during tilting but only in one of them was the renal extraction of PAH decreased and that in a very moderate way.[1] Therefore it seems safe to conclude that the circulatory changes induced in our subjects by the changes in position did not incorporate any redistribution of the circulation through

the kidneys, corresponding to a shunting of the blood from the cortical to the medullary pathways parallel to the decrease of the PAH clearance. This conclusion was also supported by the changes in the arterio-venous oxygen difference, which rather increased during tilting, instead of decreasing, which would have been the case if a shunt had been opened up. This was the case though the decrease of renal circulation in two of the cases was very marked and in spite of the marked general effect of the change in position, with fainting or near fainting as result. The absence of increased renal shunting during this procedure does not speak in

[1] In preliminary notes Bucht (Nord. Med. 1949 in press) and Josephson (Abstracts 1st Internat. Congr. Biochem. Cambridge 1949, 181) reported results indicating the presence of a renal shunt in tilting of human subjects. We now have to withdraw this conclusion as the results were due to a technical error — the PAH solution was injected through the renal vein catheter.

favour of the importance of the shunting mechanism for decreasing the blood flow through the kidney parenchyma in man, as suggested by Trueta et al. The results of the present study agree with those of Reubi and Schroeder (1949) who after adrenalin ,or histamine did not find any evidence of increased shunting through the kidneys in man. They also agree with the finding of Van Slyke (1942) in experimental shock in dogs, that the PAH extraction of the kidney did not decrease until the shock was very prolonged and severe and the renal plasma flow reduced to below 10 per cent. We could confirm the results by Brun, Knudsen and Raaschou (1945) that tilting to the upright position depresses the PAH clearance.

Of course tilting as well as the injection of drugs are much weaker damages than the crushing of legs used by Trueta et al. It is thus possible that a severe general injury may produce a deviation of the blood stream through the kidneys in man as it has been shówn to do in the rabbit. The present study only shows that smaller influences do not produce any such deviation, even if the general effect is so marked as to produce fainting. It thus seems improbable that the "Trueta mechanism" has any implications in such a disease as hypertension as has been repeatedly suggested.

The renal oxygen consumption decreased during the periods in the upright position in the two cases where the renal plasma flow decreased most markedly. In all cases there was increased oxygen consumption durin[g] the period after tilting. This was most mar ked in the case where the plasma flow in creased mostly (B. J.), but also showed u[p] in the other cases. Thus there was no ab solute parallelism between the renal oxyge[n] consumption and the renal blood flow, a[s] has earlier been shown in man during ab dominal compression by Bradley and Hal perin (1948) and during tubular excretory work by us (1949). The most obvious explanation for this is that after the tilting period with its reduced renal blood flow, the kidney tissue reacts to the prèvious state of relative anoxia that has to be compensated by an increased oxygen consumption.

SUMMARY

1. The renal extraction of PAH and oxygen was determined in four subjects at rest and during tilting to 30 or 45 degrees from the horisontal. At the same time the glomerular filtration and the total renal plasma flow were determined.

2. There was no change in PAH extraction though the renal plasma flow decreased markedly in three cases.

3. The arteriovenous oxygen difference increased a little but the renal oxygen consumption decreased in two cases during the upright position.

4. The implications of these findings are discussed, especially in relation to the findings of Trueta et al.

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ON THE TREATMENT OF URÆMIA WITH INTESTINAL DIALYSIS

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The principle of the treatment with intestinal dialysis is the simple and well-known that the lumen of larger or smaller portions of the intestines is irrigated with suitable solutions. By this means presumably noxious retention products which cannot be excreted by the kidneys can pass by diffusion into the irrigation fluid and be removed with it from the organism.

What is accomplished in this manner is a kind of substitute for filtration, and scarcely more than that; we can hardly expect to replace the function of the tubules in this manner. We know that in the tubular cells there are very effective transfer mechanisms, capable of clearing the blood, just by a single passage, of substances that have been administered, e. g. diodrast, para-aminohippuric acid, and phenol red, but we have no knowledge of the importance of the tubular cells for the excretion of the decomposition products of the metabolic processes. It is, however, not improbable that they might play a part in this excretion.

When the question is asked what are the substances producing the symptoms of uraemia and which we want to remove by our treatment with dialysis, we have no answer ready. We *know* that electrolytic disturbances play a considerable role but that they cannot nearly explain all the symptoms. We *know*

that urea hardly plays any primary part in the development of the uraemic symptoms. We suppose that the retention of substances such as phenols and guanidine plays a certain part, perhaps in conjunction with phosphates, but for the present it is generally assumed that the condition is an intoxication with many different substances about which we know relatively little. This fact is of great importance when we try to estimate the effect of treatment with dialysis, because in reality the analytical methods generally used are of little avail.

Which Parts of the Alimentary Canal may be used for Intestinal Dialysis?

The literature on the subject contains communications about the use of practically all portions of the intestinal canal. These communications show quite clearly — and in accordance with classical animal experiments — that it is possible to attain an effective dialysis only in the small intestine including the duodenum.

Technique. (Flushing the entire intestinal canal.)

The technique we have used in Department III consists simply in passing through the nose a long slender tube as far as to the beginning of the jejunum. The irrigation fluid is then allowed to pass the entire small

and large intestines and leaves the intestinal canal by a thick Pezzer catheter placed immediately above the sphincter ani. The patients have experienced very little discomfort from this procedure — indeed, in many cases they feel nothing but slight borborygmus.

The flushing liquid, in 10 litre bottles, is placed at a suitable height above the bed and, before reaching the patient, passes a water thermostat to obtain body temperature. From the rectum the fluid passes the Pezzer catheter and — via a piece of rubber tubing — on to another 10 litre bottle on the floor. Both containers are calibrated so that the quantities passed in and out can be ascertained at any time.

This technique is the simplest of all forms of dialysis that have been described. It is, however, encumbered with certain difficulties and I shall briefly refer to a few of them.

The first difficulty is to manoeuvre the tube into the right position. It sounds so simple but may sometimes be difficult in uraemic patients who vomit much. In most cases, however, it can be accomplished with a little patience.

The next problem with which we are confronted is to find a suitable irrigation fluid. However, in intestinal dialysis it is undoubtedly less of a problem than in other kinds of dialysis. Only it must be realized that in this kind of dialysis the membrane consists of *active* cells capable of performing a selective absorption, at any rate of sodium, and that the gradients of concentration are therefore not the only factors decisive for the passage of the substances through the membrane.

When Ringer's solution is used, a constant absorption of NaCl will take place quite independently of the osmotic concentration — and of the concentration in the blood, as the absorption from the intestine is quite unregulated. This will sooner or later lead to pulmonary oedema (and also produce a severe dehydration of the cells if the solution is hypertonic).

If, on the other hand, a pure solution of, for example, lactose is used, we do not in the irrigation fluid find more than about one fourth of the concentration of NaCl in the blood because of the absorption from the intestine — even if other substances, e. g. urea, have attained equilibrium in the blood and irrigation fluid. In this manner the absorptive faculty of the intestine becomes rather an advantage. It enables us to flush for some time with simple solutions of substances which are absorbed slowly or not at all without removing any large quantities of NaCl from the organism.

The passage of water through the intestinal wall is largely a passive process determined by osmotic forces. Consequently, it is possible to have hydration or dehydration of the patient under complete control by flushing with suitable concentrations of substances which are not absorbed such as, for example, lactose or sucrose. Disaccharides cannot pass the intestinal wall without being hydrolysed, and in the course of the passage of the irrigation fluid (which takes only about 20 minutes) they will not to any great extent be decomposed to monosaccharides which can be absorbed.

It should be borne in mind that if an isotonic solution of lactose is introduced into the intestinal canal, then urea, for example,

will pass by diffusion into the solution until the urea concentration is equal on both sides of the mucous membrane of the intestine. Since lactose is practically incapable of passing the mucous membrane, the solution will become hypertonic in relation to the blood and thus exert a dehydrating effect. A thorough control of the osmotic concentration of the irrigation fluid and the blood by means of freezing-point determinations is required in order to avoid any surprise with regard to the water equilibrium.

The mucous membrane of the intestine is not completely impermeable to $Mg+$ ions, and I must strongly warn against the use of $MgSO_4$ solutions as irrigation fluid without a thorough check of the Mg-concentration in the blood. We have observed perilously high Mg-concentrations in the blood after flushing for some time with $MgSO_4$, and there can be no doubt that irreparable injury may be inflicted upon the patient in this manner, as the Mg administered cannot be removed from the organism again.

Recently we have used lactose solutions *without* any admixture of Na, Cl, or HCO_3. The osmotic concentration of the solution is fixed in such a manner that it exerts a dehydrating effect, most frequently so that for each 10 litres of irrigation fluid passed down the tube, from 12 to 14 litres leave the organism. The resulting loss of fluid is made up for by intravenous administration of Ringer's or Darrow's solution diluted 1:3 with water or glucose water. This replaces water and salt in about the same ratio as they are lost through the intestine, and in this manner we achieve a withdrawal of tissue fluid and ensuing replacement with "pure" solutions.

However, it has proved practical to keep the potassium concentration in the irrigation fluid at the normal serum value (unless intended to raise or lower the potassium level in the serum). The conditions of absorption of potassium have not been elucidated in full, but it is doubtful whether any active resorption takes place from the intestine.

There are, of course, numerous problems concerning the composition of the irrigation fluid which are not easy to solve but it appears from what I have said that it is comparatively simple to control the equilibrium both of electrolytes and water.

May the Treatment be Injurious?

I may add that it cannot, of course, be avoided that a constant flushing removes important substances from the intestinal canal and that it may be difficult to supply sufficient calories during the treatment. But as it lasts only for a few days, this is presumably not of vital importance. It has never been observed that the functions of the intestine have been impaired after the treatment has been discontinued. Soon after its conclusion the stools are normal again.

The greatest demands in this form of treatment are perhaps those made on the laboratory control of the patients. It is necessary to have at one's disposal a large number of rapid methods of analysis — preferably micromethods. It is of the utmost importance to realize that *a patient suffering from anuria or almost suspended renal function is completely defenceless against changes of liquid or salt concentrations to which he is exposed during the treatment.*

Table I.

Date	Hb. %	Prot. %	Urea mg %	Uric acid mg %	Creatinine mg %	Xanthoproteine (extinct.)	NPN mg %	P mg %	K mg %	Na meq/l	Ca mg %	Mg mg %	Cl meq/l	CO₃ m mol/l	÷° ∆
1 VI		7.2	209		7.05	0.15		2.9		132					
2 »		6.8	248		9.9	0.17		2.5		133					
3 »	67	6.8	308		10.8	0.18	177	3.1		135		3.5			0.66
» »	68	6.4	331	15.5	13.2	0.17	186		14.7	139	7.1	3.8	83	28	
4 »	73	7.1	236		14.7							5.7	88		
6 »	68	6.1	262		14.4	0.23	154	4.2		134		6.3	87		
7 »	78	8.0	230									10.2	93	13	0.62
8 »	60	7.1	140	15.2	16.8	0.27	100	6.9	14.0	140	11.4	16.8	98	11	
10 »	63	6.9	90		16 2	0.30	79	8.3	10.8	139		15.0	92	12	0.54

Irrigation Fluid No.	Composition	Amount l in	l out	Urea removed g
I — VI	Mg SO₄ + K Cl	60	73.5	110.5
VII — XII	Lactose + K Cl	60	70.9	59.8

Table I shows the result of a typical experiment with the treatment. The patient was a young woman who after a severe barbituric acid poisoning developed a state of shock and, therefore, on May 30th, was given a blood transfusion — unfortunately of incompatible blood. She at once developed a subtotal anuria (24-hour output about 50 ml) which persisted for 13 days after which the excretion of urine slowly started again.

The table shows that during the first days the blood urea rose very much. In the evening of June 3rd treatment with dialysis was instituted which caused an immediate fall of the blood urea concentration. On the 4th the flushing had to be interrupted because of technical difficulties in placing the tube, and this caused the blood urea to rise again. The treatment was continued from the 6th and the urea concentration in the serum then fell from 262 to 90 mg per cent.

As for the other retention products in the blood: uric acid, inorganic phosphate and the xanthoproteic index it appears that they show constant values (uric acid) or a rise during the treatment, presumably indicative, among other things of a low permeability of the mucous membrane for these substances.

The Mg-concentration showed a very considerable rise, however, no injurious effect of this was observed; flushing was continued with lactose, but this caused only an insignificant fall of the Mg-concentration.

Na, Cl and CO₂ showed low values but for fear for producing intrarenal oedema we desisted from a correction before the excretion of urine had been started again.

The freezing-point depression of the serum fell to the normal value during the treatment.

The lower part of the table shows the amount and nature of the irrigation fluids used, and the quantity of urea removed, 170 gm in all. It is seen that the amount of irrigation fluid leaving the organism is considerably larger than the one passed down the tube and that a total quantity of 24.4 litres of liquid was removed and replaced in the course of treatment by a similar quantity of "interstitial salt solution" (Darrow(1)).

On June 10th the secretion of urine started slowly again and dialysis was therefore discontinued. This was followed by transient rise of the

blood urea, but the patient was disharged feeling completely well with a renal function somewhat less than half the normal.

There were no clinical signs of uraemia at any time during the entire course.

The conclusions that can be drawn as to the effect of the treatment with dialysis are as follows:

1) It is possible to control the water equilibrium in the course of intestinal dialysis in such a manner that great changes can be avoided, or — if desired — considerable oedemata can be removed.

2) By varying the content of sodium and potassium, chlorine and bicarbonate in the irrigation fluid and in the liquids given by parenteral administration, the electrolyte composition of the organism can be arbitrarily changed — the concentration of the ions can be either *increased* or *lowered.*

3) Urea passes readily by diffusion through the mucous membrane of the intestine and can therefore be removed from the organism by intestinal dialysis.

4) With regard to the ·concentration of retention products other than urea and NPN, there is a rise of all the substances that have been determined. (This is seen in almost all experiments, except in a patient who was treated with Ringer's solution — a matter of simple dilution of the extracellular fluid.)

5) As to the *clinical effect,* I must say that in some cases it is undoubtedly present but may perhaps be attributed to simultaneous blood transfusions or corrections of electrolytes; in other cases it is undoubtedly *absent.*

It may perhaps be considerd that an experiment such as the one accounted for in Table I is a proof of the efficacy of the method. To live for 13 days with anuria without becoming uraemic is — or has for many years been — a rare occurrence. Observations have, however, been published which seem to show that if the patients are not overloaded with water and electrolytes, whilst at the same time their energy requirements are covered by means of fat and carbohydrates, it is possible in anuria to obtain just as long, and still longer, periods of survival as in the case of a more active therapy.

The results from the Hammersmith Hospital(2), recently published in the Lancet, and similar results from Texas(3) show that the method of treatment which at present must be recommended in general in the treatment of acute uraemia must absolutely be the high caloric regimen poor in liquid, electrolytes and nitrogen.

If the promising results of the conservative treatment hold, so that a patient suffering from anuria can live on this treatment for about 3 weeks, the scope of indication for active dialysis therapy will undoubtedly be considerably restricted. The chances that injured kidneys will resume their function after more than 3 weeks' anuria can at any rate hardly be very great.

It cannot yet be decided whether intestinal dialysis is effective, and there is every reason to continue the investigation of this problem, but it is absolutely too early to take the method into use outside departments where a special interest is taken in it.

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For literature concerning intestinal dialysis see

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THE PATHOLOGY OF GLOMERULONEPHRITIS
AND RELATED DISEASES

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Owing to limited space we must restrict this account to one or two groups of kidney disease which are of particular interest at the present time.

Volhard and *Fahr*, whose classification of kidney disease has been commonly used during the last thirty years, interpreted glomerulonephritis as a separate disease which they divided into three stages, the acute, the subacute or subchronic, and the chronic stage. During the second stage the disease may present a nephrotic syndrome.

A group of English investigators led by *Ellis* has recently put forward a new classification. According to them Volhard's and Fahr's "nephritis with nephrotic syndrome" constitutes a special disease which they call glomerulonephritis type 2 to distuinguish it from glomerulonephritis type 1.

Nephritis type 1 is always preceded by an infection of haemolytic streptococci and is generally considered as being an "allergic reaction". The symptoms appear suddenly and indicate capillary damage with haematuria as most striking phenomenon. In general recovery is rapid and only in a small number of cases does a chronic disease develop.

Type 2 on the other hand has the character of a dyscrasic disease, and the onset is insidious. As is well known, the initial symptoms are edema, abundant albumin in the urine, and diminished protein content of the blood; but no haematuria. The blood pressure is generally normal, but may sometimes rise considerably in the last stages of the disease. The prognosis is poor even though complete remissions for several years may often occur. Addis does not regard this disease as a glomerulonephritis.

The histology undoubtedly supports the theory that glomerulonephritis types 1 and 2 are two different diseases and that the former is of an "allergic" nature.

The histology of "allergic" damage of the capillaries of the lungs and of the brain has been described by Rich and H. Bergstrand in different forms of allergic disease. It is characterized by focal fibrinoid necroses of the capillary walls, enlargement and proliferation of the endothelial cells and fibrin thrombi in the lumina. Similar changes are seen also in the capillary tuft of the glomeruli and sometimes in the art. afferens in the acute and subacute stages of glomerulonephritis type 1 (Fig. 1). They can also appear simultaneously in other capillary areas in the body as we were able to demonstrate in a few cases in the lungs and brain (Fig. 2).

As for vessels of larger caliber, it is well known that changes similar to the "allergic" changes in periarteritis nodosa are in many cases observed in the arteries of kidneys in acute glomerulonephritis. In other words the combination of periarteritis nodosa and glomerulonephritis is remarkably common. In this connection may be mentioned the peculiar glomerular damage sometimes accompanying periarteritis nodosa called Wegener's granulomatosis and closely resembling the picture seen in glomerulonephritis.

Another illness which should perhaps be included in the group of "allergic" kidney diseases is bilateral cortical necrosis of the kidneys. About fifty per cent of the cases described have occurred in pregnant women and the disease has therefore been associated with toxaemia of pregnancy or with the haemorrhagic conditions of pregnancy. In a large number of cases, however, the condition is preceded by an acute infection in no way connected with pregnancy, and Dunn considers that these are cases of an extremely acute glomerulonephritis. Three out of the four cases we have observed were due to an acute infection. In these cases proliferation of the endothelial cells was observed with fibrin thrombi in the lumina and necroses of the walls of the glomerular capillary tuft and in the afferent artery. The changes even extended to vessels the size of the interlobular artery which explains the extensive necrosis in the kidney cortex. Small fibrin thrombi may be observed in the vessels of other organs in this disease, but they are insignificant in comparison with those of the kidneys.

In glomerulonephritis type 2 the changes in the glomeruli in the initial stages have a different appearance. At first they are quite insignificant and there is only a moderate thickening of the basal membrane of the capillary network (Fig. 3). This thickening proceeds so that, on transverse section, the capillary wall gradually comes to look like a hyaline ring with a more or less narrowed lumen. Finally a hyaline ball forms which may be entirely devoid of patent lumen. The changes, however, are not uniform in all the capillaries, and the balls just described are usually separated by wide, more or less well preserved capillaries. Alongside the glomerular changes there occur severe changes in the proximal and distal convoluted tubules. They are dilated and lined either with the original epithelium or with a low regenerated epithelium and usually contain hyalin droplets, neutral fat and doubtly-refractive cholesterol esters. Fat is also seen in macrophages in the interstitial tissue which is distended with edema.

The changes in type 2 are more diffuse than in type 1. In the advanced stage, when shrinking occurs due to the diminution of the kidney parenchyma, the surface is comparatively smooth, even though the kidney is reduced in size, in comparison with tfhe granulated surface of the kidney in chronic glomerulonephritis type 1, or in arteriolosclerosis. In the shrinking stage, however, these three forms may be difficult to differentiate anatomically.

Glomerulonephritis type 2 is, in our opinion, equvalent not only with Volhard and Fahr's "glomerulonephritis with nephrotic syndrome", but also with what these authors call genuine nephrosis and what Epstein described under the name of lipoid nephrosis in children. Cases which had been previously diagnosed as lipoid nephrosis on

Fig. 1. Woman, 44 years. Chronic otitis media. Operated on 31/7 1946. Tiredness and loss of appetite. 28/10: temperature 38.5° and macroscopic hematuria. Edema of the eyelids but not of the legs. Blood pressure 140/80. Very little albumin in the urine. Rapid deterioration and death 7/11 without definite cerebral symptoms or uremia.
Enlarged glomerulus with swollen endothelial cells. Small fibrinoid necroses in the capillary walls and fibrin thrombi in the lumina. Red blood corpuscles in the capsular space. Proliferation of capsular epithelium.

Fig. 2. Same case as Fig. 1. Autopsy revealed multiple small hemorrhages throughout the white matter in both cerebral hemispheres.
Necrosis of the capillary wall with a fibrin thrombus in the lumen. Necrosis of surrounding cerebral tissue and a fresh ring-shaped hemorrhage round the necrosis.

Fig. 3. Man, 52 years. Treated for a fracture of the tibia Jan 1947. Routine examination of the urine revealed proteinuria. Esbach 12 per cent. Sed: moderate amount of red and white cells and hyaline casts. Bloodpressure 130 75. Serum

protein: albumin 1.48 per cent, globulin 1.31 per cent, fibrinogen 1.12 per cent. Blood sugar 1... N. P. N. 39 mg per cent. Treated at home unt... March 1949. Continuous proteinuria. During t... last two months dyspnoea, cough and edema the legs. Oliguria. Blood pressure 160/90 N. P.... 305. Death 12/3 in uremia.
The kidneys were shrunken weighing 220 g t... gether. Hyalinisation of the basement membra... of the glomerular capillaries. The tuft seems be "lobulated" and adheres in places to the capsul... No proliferation of the capsular epithelium.

Fig. 4. Boy 4½ years. 11 months previous to h... death he developed sudden severe general edema an... very large quantities of albumin in the urine. R... mittent course with short intervals from symptom... No hematuria, no rise in blood pressure nor in... crease in the blood urea. Died of facial erysipela... Enlarged smooth pale kidneys, combined weigh... 380 grams. Convoluted tubules enormously dilate... with low epithelial cells which contains masses o... doubly-refractive fat. The glomerular capillarie... show focal hyalinisation of the basement membran... Partial fusions of the capillaries and adhesions t... the capsule. The capsule shows no changes. (Cf. fig. 3.)

account of their typical clinical and anatomic picture, have on re-examination been proved to present changes in the glomeruli of the type which characterize the initial stages of glomerulonephritis type 2 (Fig. 4).

In common with a number of other authors we have not been able to find any fundamental differences between Kimmelstiel-Wilson's glomerulosis and glomerulonephritis type 2. According to Kimmelstiel-Wilson these kidney changes should only occur in diabetes with hypertension, but it has since been necessary to abandon this conception. Certain authors hold that the clinical picture in glomerulosis and nephritis type 2 is different, but the difference seems to be immaterial (Porter-Walker, Hilden). Allen states that it may be possible to im-

pregnate with silver the hyaline masses in the glomeruli in glomerulosis but not in nephritis. We have found a positive reaction only in the very advanced cases of glomerulosis.

In this connection we would like to point out the clinical and anatomic similarity between glomerulonephritis 2 and amyloid nephrosis. Edema, large quantities of albumin in the urine, altered protein content of the blood and blood pressure rising first in the shrinking stage, are common to both condition. Multiple myeloma and, to some extent, even eclampsia present similar symptoms. It appears therefore as if we were dealing with a special group of diseases with certain common features.

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2

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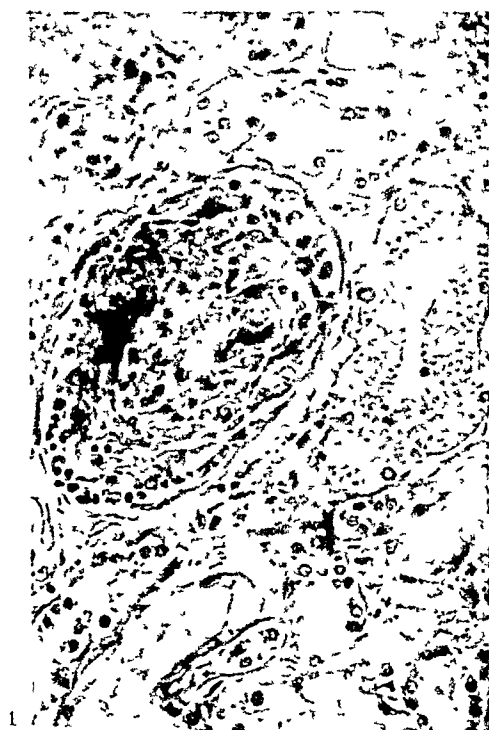
Anatomically they are characterized by, hyaline droplets in the kidney epithelium which indicate a disturbance of the protein metabolism. It is now fairly generally agreed that the hyaline droplets do not indicate a degeneration but are due to changes occurring in the epithelium in connection with reabsorption of protein (Randerath, Oliver et al.). Even the "fatty degeneration" of the kidney which so often arises in these diseases is interpreted in the same way. It is an open question how the appearance of pathological protein in the tubules can' be explained. Bence-Jones protein, with its low molecular weight, can undoubtedly pass through intact glomerular capillaries. On the other hand it is difficult to believe that the intact capillary would permit the transmission of the high molecular protein material which appears in the blood, for instance in "amyloid nephrosis". We must therefore assume that damage to the capillaries of the tuft in some way occurs in connection with the primary metabolic disturbances (Randerath, Zollinger et al.) eventually causing a hyalinization of the basement membrane.

It is worth noting that certain amino acids also appear to be able to damage the kidneys. They may either result from a metabolic disturbance as in the Debré — de Toni — Franconis syndrome or be brought about experimentally. The kidney changes in this syndrome are also · characterized by hyalinization of the glomerular capillaries and the appearanc of hyaline droplets in the epithelium, dilatation of the tubules etc. Looser therefore compares the changes with those of Kimmelstiel-Wilson's disease.

Finally a word on the clinical picture which in American and British literature is called "lower nephron nephrosis", but which we prefer to call "distal tubular nephritis" on account of the inflammatory reaction which usually accompanies the damage of the tubules. The clinical picture is now well known and is met with in conditions which cause haemolysis or, with the occurrence of myoglobin in the blood, and with the use of a number of drugs (which do not give rise to haemolysis) especially sulphonamides. Degeneration and necrosis of epithelial cells in the distal part of the nephron are found which may be so extensive that the wall of the tubules is disintegrated in places. Around such a centre a strong inflammatory reaction occurs which may break through to an adjacent thin-walled vein so that a tubulo-venous communications arises. In the tubules are found casts with pigmented granules of haemo-derivatives or, in cases of sulphonamide poisoning, crystals of the drug. In many of the tubules active regeneration of the epithelium is seen. According to H. Bergstrand this inflammatory process is also a cause of scar formation in the kidney.

With regard to the pathogenesis, opinions are very divided. It has been thought that the casts in the tubules would obstruct the passage of urine and in this way give rise to anuria, but this theory has had to be abandoned. Others have thougt that the broken down haemo-derivatives or the drugs may cause direct damage to the epithelial cells especially if the urine is acid. Thus Bing has shown that methemoglobin has a damaging effect on the kidney epithelium of dogs if the urine is acid, but not if it is alkaline. But the syndrome has also been observed in man with alkaline urine, for instance in cases of incompatible blood trans-

fusions. Some investigators have held that the patients are always in a condition of shock and therefore consider that hypoxaemia of the kidneys plays a significant role in the origin of distal tubular nephritis.

The investigations of Yuile et al. indicate that hypoxaemia and acid urine make the epithelial cells more susceptible to the toxic effect of the haemoderivates and drugs.

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$\mathscr{A}bstracts$

GLIMSTEDT (Institute of Histology, University of Lund, Sweden): S t r u c t u r e   a n d
H i s t o c h e m i s t r y   o f   t h e   N e p h r o n.

The structure of the glomerulus permits a fairly
curate interpretation of its mode of function.
ich is not the case, however, with the other parts
the nephron and the collecting tubules. The
:tremely varied structure of these portions indi-
tes a complicated function. The main charac-
ristic of the proximal convoluted tubule is the
)er-shaped outgrowths on the apical surface of
e cells. These outgrowts have been interpreted
; a striated border analogous to that of the epi-
ielium of the small intestine. Quite naturally, the
'oximal convoluted tubule was believed to have
resorbing function. Recent investigations have
:monstrated, however, that the fiber-shaped out-
rowths are, not a striated border, but a real brush
)rder, and thus not analogous to the striated
)rder of the epithelium of the small intestine.
he parallel rods of the cells in the proximal and
istal convoluted tubules must be considered as
:creting cellular organoids. Since the proximal
onvoluted tubule consists of four different por-
ons (Sjöstrand), histophysiologic research must
over these segments as well. Studies of the Golgi
pparatus as well as vital stainings and fluor-
scence microscopical investigations indicate re-
orbent processes in the proximal convoluted tubule.
;tructural conditions and morphological experi-
nental investigations thus seem to indicate a double
ôle of the proximal convoluted tubule with secre-
ory and resorbent functions.

In the other parts of the nephron and the col-
ecting tubules there are specialized structures, the
unctions of which are as yet unknown. It has,
herefore, seemed desirable to work out a technique
)f investigation which facilitates an analysis of the
:elationship between cellular structure and cellular
unction. In accordance with principles previously
pplied to other organs by Linderstrøm — Lang
ind co-workers, a renal cylinder is removed, and,
following histo-technical treatment, is embedded in

electrolyte-free paraffin.[1] Sections cut by a micro-
tome are taken alternately to ultramicrochemical
and quantitative histological analyses. By corre-
lating the determined chemical substance with the
various nephron parts, the desired relationship be-
tween structure and function is obtained. The use-
fulness of the method was demonstrated in the
lecture by a description of the distribution of the
chlorides in the kidney. About two-thirds of the
chlorides which are filtered away in the glome-
rules are reabsorbed in the proximal convoluted
tubules (Walker, Bott, Oliver,- and McDowell).
Thus, a coarse regulation takes place here. The
investigations submitted all showed that the col-
lecting tubules perform an active part in the
mechanism of urine production. In the collecting
tubules there is, for example a fine-regulation
mechanism coupled to "cellules spéciales" (Okkels)
which maintain the blood-chloride concentration
at a normal level under varying conditions.

For a more detailed report on this subject the
reader is referred to the following:

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B. VIMTRUP (Institute of Pathology, Bispebjerg
    Hospital, Copenhagen):   H i s t o l o g i c a l
    E x a m i n a t i o n s   o f   K i d n e y s   o f   H e t e -
    r o m y i d a e.

During recent years Drs. Bodil and Knut
Schmidt-Nielsen have carried out extensive investi-
gations on the water economy of certain species
of heteromyidae living in desert, viz. dipodomys

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[1] I am indebted to Prof. Sune Bergström for
the preparation of this paraffin.

spectabilis (bannertailed kangaroo rat), dipodomys desertii, dipodomys merriami and perognathus bayleyi (pocket mouse). These animals live on entirely dry food and must conserve water by every possible means. They excrete urine with a maximum concentration of 1.1 N for electrolytes and 3.8 M for urea, i. e. about 3 times the maximum concentration of human urine. Histological examinations of the kidneys of dipodomys spectabilis and deesertii undertaken during the winter of 1949, reveal that the distal tubule is of considerable width, its lining cells are large, domeshaped and have vacuolized cytoplasm near the lumen, while there are moderate numbers of plastosomes and rod-like mitochondria in the basal part of the cell. The nucleus is rounded with fine granules of chromatin and a fairly rich amount of fluid. The proximal part of the collecting duct is lined with cuboidal cells. In the outer zone of the pyramid the cells are flattened, the oval nuclei projecting towards the lumen. In the inner zone there are transversely arranged cells with fine fibrils, that probably encircle the ducts. The nature of these cells is yet unknown. Intravenously injected indigocarmine appears as small granules in the proximal part of the nephron and as coarser granules in the primary collecting ducts. In the terminal part of the collecting duct the dye is accumulated into dense masses which almost entirely block the lumen and look like concrements. This seems to indicate that the urine is heavily concentrated through its passage down the collecting ducts. I have not been able to observe any resorption of water through the wall of the bladder.

H. KOEFOED (Institute of General Pathology, Copenhagen): Investigation of the Kidneys of the Kangaroo Rat by the Maceration-method.

As in most other kidneys there were found two maintypes of nephrons: the peripheral ones with a short loop of Henle and the more central ones with a long loop of Henle. The proportion between the number of peripheral and central nephrons was found to be almost the same as in other rodents.

The proximal tubules form a relatively shorter, and the thin segment a relatively longer part of the nephron than is the case in other rodents, and this agrees with the findings by I. Sperber in other desert-rodents. The collecting tubules do not form arcades but join directly.

There is nothing in which these kidneys differ from other rodent-kidneys except that the collecting tubules in a certain zone have different structure. This zone is situated near the boundary between the cortex and the medulla, is found on all collecting tubules and is of about the same length on all of them. The collecting tubule is here very thin and transparent and seems to consist of a layer of very flat epithelium cells.

J. BING and G. TEILUM: (University Institutes of General Pathology and Pathological Anatomy, Copenhagen): Effect of Intraperitoneal Injections of Protein Hydrolysates on Rat Kidneys.

In an earlier publication (Acta path. et microbiol. Scandinav. 23, 540, 1946) it was shown that after injecting rats intraperitoneally with alkali-hydrolysate of serum protein or casein, pronounced tubular changes develop in the kidneys. In continued investigations (which will be published in extenso in Acta path. et microbiol. scand.) it has been shown how these changes could already be seen after 24 hours and how they increased proportionately with the number of injections, the injections being continued for up to three months, when widespread vacuolization was found in tubuli contorti I. When the injections are discontinued the changes disappear slowly, vacuoles still being observable after four months. With a less hydrolyzed preparation the changes were less pronounced and lay in a more central zone of the kidney.

The average kidney weight of the injected animals was higher than that of the controls. The percentual dry-substance, nitrogen and phosphatase contents of the pathological kidneys were lower than those of the controls, but owing to the increased weight of the kidneys the total contents were found to be slightly increased for dry-substance and nitrogen, and equal for phosphatase.

Blood urea and serum proteins were found to be unchanged after the injections.

O. TORGERSEN (Institute of Pathology, Rikshospitalet Oslo): Further Studies on the Morphology of Pre-urine in Situ.

Cytology, strictly speaking, deals only with the structure of the cells. The microscopist is, however, not seldom concerned with the study of structures which apparently are situated outside the cells, especially if these structures appear as solid masses, e. g., intercellular substances. In some instances, even liquid or semiliquid substances may be visible in microscopical sections, e. g., the "colloid" of the thyroid or, in some instances, edema fluid. The reason why these fluids are not dissolved by the process of fixation, embedding and staining is not known, although it would seem reasonable to suppose that their protein content might be an important factor. Previous studies on the morphology of the renal tubular contents have not given unequivocal results. The reason for this may be that unsuitable fixatives and staining methods have been employed. In a previous publication (1) reference was made to a combined fixation and staining method which acts in the course of ninety minutes or less and in which no

water or other fluid solvent is allowed to enter in contact with tissue before the fixation-staining is completed. The reagent employed was osmium tetroxide vapour which, apart from its capacities as a fixative, is reduced (blackened) according to the different reducing properties of the various substances present in the cells and in the "visible" extracellular fluids. As such substances were found apparently within the lumen of the proximal convoluted tubules and not in the more distally situated parts of the nephron, it would seem reasonable to suppose that the reducing substances had been reabsorbed in the proximal parts. If this explanation is correct the presence of reducing substance in the distal parts of the nephron might be anticipated if tubular reabsorption is interfered with experimentally. In animals given corrosive sublimate or uranyl nitrate (even in does not sufficient to cause albuminuria) and in experimental hydronephrosis, large amounts of reducing substance were found in the distal convoluted tubules and in the excretory ducts. This indicates that the problem of renal tubular reabsorption may be approached by histological methods.

Another rather startling observation was that the various types of cells, and the pre-urine present in different parts of the nephron, reacted very differently to physiologic saline or to isotonic glucose solutions. For instance, the contents of the glomerular capsule and of the proximal convoluted tubules showed a very considerable swelling when small pieces of kidney tissue were immersed in isotonic glucose solution for one hour prior to fixation. This swelling could be prevented only by increasing the concentration of the bath to about four times the isotonic value. This may appear to be an interesting parallel to the reaction of mitochondria under similar conditions. It strongly supports the conception that glomerular filtrate contains either albumin or another colloid substance which is reabsorbed in the proximal convoluted tubules under normal conditions.

These questions will be fully dealt with later, when the investigations are completed.

Literature:

Torgersen, O.: Studies on the Morphology of Renal Tubular reabsorption. Proceedings of the 6th International Congress of Experimental Cytology. Stockholm 1947.

F. LÖFGREN (Institute of Anatomy, University of Lund, Sweden): The Topographical Arrangement of the Malpighian Pyramids in the Human Kidney.

The embryonic ureteric tree comprises principally seven paired branch areas with a ventral and dorsal component in each pair. This system can be divided into two main groups: an upper

group corresponding to the primary cranial lobe of the kidney and comprising the first three pairs of branch areas, and a lower group corresponding to the primary caudal lobe of the kidney and comprising the last four pairs of branch areas. This latter group can be subdivided into two smaller groups each with two pairs of branch areas.

The branch areas of the ureteric tree constitute the matrix for the subsequently formed pyramids and the topographical arrangement of the pyramids is, therefore, determined by the manner in which the ureteric tree is differentiated. In the later embryonic as in the adult kidney we also find three pairs of pyramids above the hilum (pars cran.) two pairs in the level of hilum (pars intermedia) and finally below the hilum two pairs (pars caud.). This condition is confirmed by reconstruction in wax ad modum Born of the whole pyramid-system from 20 adult kidneys.

The pyramids can be more or less fused with another. The average total number of papillae is 8.68 ± 0.17.

In the embryonic kidney the pyramids determine elevations on the exterior of the kidney, colliculi which agree essentially in number and position with the branch areas of the ureteric tree. The exterior of the adult kidney usually shows more or less persistence of the fetal lobulation, and the topographic system of the pyramids therefore can be outlined from the surface of the kidney.

Usually the hilum is directed more or less dorsally in the right kidney but ventrally in the left kidney. This fact depends on the almost regularly occurring displacement in a medio-lateral direction between the ventral and dorsal halves of the kidney. In the same way the column of Bertini, running longitudinally between the anterior and posterior row of pyramids, assumes an angular form in cross section. At the same time the boundaries between the dorsal and ventral vessels become complicated to a very high degree.

According to the original duality in the anatomic organisation of the kidney the pelvis has a primary angle of bifurcation which, however, only in rare cases coincides with the main angle of the pelvis.

M. SIMONSEN (Institute of General Pathology, Copenhagen): Endocrine Kidney.

"Endocrine kidney" is a term introduced by H. Selye for a kidney whose exocrine function has ceased but whose endocrine secretion of pressor substances is increased. It is produced in rats by a modification of Goldblatt's method: the aorta is constricted with a silk ligature between the departures of the two renal arteries, of which the left one takes off slightly distal to the right. By this means the left kidney becomes relatively ischaemic and hypertension develops, and simultaneously filtration ceases owing to reduced pres-

sure in the glomeruli. Selye states that thereafter the glomeruli disappear whereas the distal third of the tubuli contorti I proliferate, and he concludes from this that the pressor substances are hormones formed in the cells of the proliferating tubules.

I have endeavoured to reproduce Selye's findings, carefully adhering to the mehod described. In 12 cases the rats developed malignant hypertension diagnosed by the criteria of fibrinoid necrosis in the vessels + cardiac hypertrophy. In 5 cases there was benign hypertension, the criterion being cardiac hypertrophy without vascular necrosis. In 5 other cases there was pronounced atrophy of the left kidney without cardiac hypertrophy or noteworthy vascular changes. If the pressor substances are to be regarded as hormones, the left kidney of the first 17 rats must be said to have unfolded endocrine activity; but in only one case — a moribund rat which was killed 13 days after operation, was there a histological picture of the left kidney identical with Selye's microphotographs. In the other 16 cases the changes in the ischaemic kidney were of the same kind as those usually found in "Goldblatt kidneys", while the changes in the heart were of the same nature as when the left kidney satisfied Selye's morphological criteria for endocrine activity. In fact, the histological changes in the heart were more considerable in several cases of ordinary "Goldblatt kidneys" than in the case with "endocrine kidney".

Selye's criteria for proliferation of the tubular epithelium are: 1) papilliform proliferation in the otherwise collapsed, solid tubules and 2) mitoses in these proliferations. After comparing my own findings with Selye's photographs I must contradict Selye's histological interpretation. In both cases the tubules are characterized by degenerative changes in protoplasm and nuclei. Most of what Selye describes as active proliferation of tubular cells must be regarded as cylinders which have been thrown off from the outermost, highly atrophic layer of the cortex and wedged themselves firmly in distal parts of tubuli contorti I, whence further passage is obstructed by the thin limbs of Henle. These coalesced cell accumulations which resemble papilliform proliferations because they are often adjacent to one side of the tubule wall, naturally show still greater nuclear atypy than the tubule cells still in situ, as they must be still more poorly nourished than the latter. For this reason, degenerate nuclei of mitotic appearance are more frequent luminally than peripherally in the tubules. I shall not deny the reality of some of Selye's mitoses, but recall the fact that most cells in the organism are capable of proliferating secondarily in relation to a primary injury. Even under pronounced ischaemic conditions this was observed on transplantation of renal tissue 32 years ago (L. Loeb). Selye's observation of vascular changes proximal

to the aorta constriction resembling periarteritis nodosa can be confirmed.

Conclusion: The "endocrine kidney" is a histological curiosity among atrophic "Goldblatt kidneys" and bears greater morphological and pathophysiological resemblance to an exocrine gland in degeneration than an endocrine gland in proliferation.

(To be published in Acta path. scandinav.)

T. BJERING (Kommunehospitalet, Copenhagen): Measurement of Glomerulae Filtration Rate.

An account is given of the value of the 3 most important substances employed for filtration measurements: inulin, mannitol, and sodium thiosulphate. It is pointed out that the inulin clearance gives the most reliable measure for the filtration, whereas one should be wary of using mannitol. In cases of nephropathy both inulin and particularly mannitol may diffuse back through the tubules, whereby the filtration determination is rendered unreliable. The thiosulphate clearance, the determination of which offers considerable technical advantages, still needs further investigation before the question of its applicability can be finally settled.

Published in Ugeskrift for Læger.

T. BJERING (Kommunehospitalet, Copenhagen): Renal Excretion of Urea.

Our present knowledge of the renal excretion of urea is discussed. While the first stage of urea excretion — the glomerular ultrafiltration — must be regarded as sufficiently proved, the laws for the reabsorption of urea are still obscure. Some of the literature available is reviewed, and it is pointed out that a simple diffusion of urea through the tubules dependent exclusively on the concentration gradient cannot explain the many different observations. The tubular membrane does not seem to be particularly permeable to urea. Other factors, still unknown must be supposed to have a regulating influence on the reabsorption of the urea.

Published in Festskrift til Poul Iversen. Suppl. to Acta med. Scand.

Discussion:

C. BRUN: Identity has most recently been ascertained between the clearances for allantoin and and inulin in man; the allantoin determination is, however, still so complicated that the substance cannot be employed in clinical work as a measure of the glomerular filtration.

The investigation of a group of compounds — polyethylene glycols — which can be made with

a molecular weight from 400 to 6000 as desired, is very promising. It has been shown that polyethylene glycols with a molecular weight under 1500 have a clearance which equals the filtration. The clearance decreases with higher molecular weights. These substances seem further more to render possible an estimate of the size of the pores of the glomerular membrane, but they, too, are difficult to determine and not yet suited for ordinary determinations of the filtration.

Dr. Bjering wanted an examination of the suitability of thiosulphate for determination of the filtration in patients with diseased kidneys. In a group of 48 patients with ultrafiltrations varying from 3 ml per minute to completely normal values I have found the ratio between the thiosulphate and inulin clearances very nearly equal to 1.0. The identity between the clearances was independent of the degree of renal injury and the nature of the disease. The examination is published in Acta Med. Scand. Suppl. *234*, 63, 1949.

E. BLEGEN, H. N. HAUGEN and K. AAS (Ullevål Hospital, Department Krohgstøtten, Oslo): Endogenous "Creatinine" Clearance.

In 37 normal individuals (67 clearance periods) the average endogenous "creatinine" clearance was 105 ml per minute ± 21 per 1.73 m² surface. Simultaneous determinations showed that endogenous "creatinine" clearance in 40 normal individuals (51 clearance periods) and 30 patients with valvular heart disease (54 periods) was about 60 per cent higher than urea clearance. In 36 patients with renal disease the endogenous "creatinine" clearance was about 90 per cent higher than urea clearance. There was a good correlation between the two values. For practical purposes endogenous "creatinine" clearance has considerable advantages as compared with urea clearance. The level of the plasma concentration of endogenous "creatinine" clearance will give the clinician a fairly good idea about the extent of the kidney lesion.

Simultaneous determinations showed that endogenous "creatinine" clearance in 37 normal individuals (103 periods) was 16 per cent lower than inulin clearance. In 27 patients with valvular heart disease (103 periods) the endogenous "creatinine" clearance was 11 per cent lower than inulin clearance. In 13 cardiac patients + 2 normals (85 periods) it was also 11 per cent lower than thiosulfate clearance. There was a good correlation between endogenous "creatinine" clearance and inulin- or thiosulfate clearance. The results establish more firmly the idea that the *real* endogenous creatinine clearance is identical with the glomerular filtration rate.

Published in Scandinav. J. Clin. & Lab. Investigation. nr. 3, 1949.

P. EFFERSØE (Institute of General Pathology, Copenhagen): Comparative Investigations into the Inulin, Creatinine and Thiosulphate Clearances of Rabbits after Ingesting Sodium Benzoate.

Bronfenbrenner and Favour have shown that benzoic acid can diminish the elimination of penicillin, probably by reducing, the secretion of penicillin in the tubules after a conversion to hippuric acid.

The author has made simultaneous determinations of the inulin, creatinine and thiosulphate clearance of 10 rabbits and found that benzoic acid also has an effect on the barrier-function of the tubules vis-a-vis some of the filtration substances. The clearances of the three substances are identical before the rabbits receive the benzoic acid, but after the injection of ½ to 1 g of sodium benzoate intravenously the creatinine clearance may fall to almost the half (average 80 per cent) and the thiosulphate clearance to 85 per cent (average 92 per cent) of the inulin clearance. The fall of the creatinine clearance in relation to the inulin clearance differs from one animal to another, but the fall of the creatinine and thiosulphate clearances in relation to the inulin clearance is parrallel in the different rabbits.

The fall of the creatinine and thiosulphate clearances in percentage of the inulin clearance can be explained best by assuming that the tubules have been so injured that the creatinine and thiosulphate are diffused back. It cannot be decided whether or not inulin also diffuses back, as the inulin clearance is not stable in rabbits.

The benzoate or one of its conversion products in the organism does not disturb the analysis of the clearance substances, for they are all recovered in full if the rabbit is given the usual dose of sodium benzoate and known quantities of clearance substances are added to its urine and plasma.

The effect on the tubules is not fatal, as the ainmals are able to live more than a month after the test and in good general condition. Uranium intoxication produces degenerative changes in the tubles and in these cases it was found, that creatinine diffuses back through the damaged tubules. After the administration of benzoate, however, it was not possible to find microscopic changes in the tubles so that the changes are therefore merely functional.

P. EFFERSØE (Copenhagen): On the Effects of Filtration and Concentration on the Backdiffusion of Urea in the Tubulus of Rabbits. Will be published in extenso in Acta physiol. scandinav.

C. BRUN (Medical Department III, Kommune-hospitalet, Copenhagen): The Influence of Renal Function Tests on the Function of the Kidneys. (Preliminary Report.)

However fundamental it may seem, the question whether the modern renal function tests themselves influence the function of the kidneys has been the subject of very few examinations.

As it has been recently communicated (1, 2, and 3) that changes of the filtration and the renal blood flow have been observed in clearance and Tm-experiments we can hardly avoid a closer examination of this question.

The susceptibility of the renal function to a great variety of *external and internal* factors is discussed. The importance of pyrogen free solutions and utensils is pointed out. Special attention is paid to the manner in which the patients are waterloaded before and during the experiments. Heavy waterloads — especially in connection with large injections of osmotic active substances such as diodone, PAHA and thiosulphate — are very apt to produce malaise in the patients and to lower their filtration rate and renal blood flow.

Consequently it is advisable to work with a *moderate waterload of the patients* and with *slow injection* of such *small quantities of the substances* as are analytic warrantable.

Personal examinations.

The experiments were made by determining the urea clearance, diodrast clearance, endogenous creatinine clearance and, in some cases, the inulin clearance in a patient in 3 preliminary periods; these were followed by injection of thiosulphate, and the same clearances where then followed for 3 or more periods under continued injection of thiosulphate.

The concentrations of thiosulphate were the same as are generally used in clearance determinations (about 50 mg per cent).

Nine experiments were made, showing that in a few cases the injection of thiosulphate was followed by considerable variations of the clearances examined, but that generally only a slight change took place (most frequently a fall).

Three of the patients complained of slight malaise (nausea), but no great discomfort was observed.

It was difficult to obtain entirely ideal solutions of sodium thiosulphate, even when boiled-out CO_2-free water and cautious sterilization were employed and it is not quite impossible that a number of the changes observed are due to inferior thiosulphate preparations.

Attempts to make more suitable injection preparations are in progress and I intend to continue the investigation of this problem which is of fundamental importance for the determination of the filtration rate by means of Sodium thiosulphate.

References:

1) Lippmann, Richard W.: Simultaneous clearance determinations in the rat. Am. J. Physiol. *155*, 282, 1948.
2) Eggleton, M. Grace and Y. A. Habib: Action of thiosulphate on the kidney of the cat. Nature. *163*, 1000, 1949.
3) Crawford, Betty: Depression of the exogenous creatinine-inulin or thiosulfate clearance ratios in man by diodrast and p-aminohippuric acid. J. Clin. Investigation. *27*, 171, 1948.

B. JOSEPHSON (Central Clinical Laboratory S:t Eriks Hospital, Stockholm): The Mechanism of Renal Tubular Excretion.

When diodrast was injected intravenously into rabbits, the iodine concentration in the kidneys reached its maximum after about 10 minutes and then slowly decreased rather parallel to the plasma concentration. If caronamide or a mercury diuretic was given before the diodrast, the iodine did not reach the same concentration in the kidneys of these animals as in untreated ones. Thus caronamide and mercury diuretics seem to hamper the ability of the excretory tubular cells to take up diodrast. If caronamide or a mercury diuretic was first given when the diodrast concentration in the kidneys was at its maximum, the disappearance of the diodrast from the kidneys was slower than normal in the caronamide-animals but not in the mercury-animals. Thus caronamide, but not the mercury diuretics, seems to make it difficult for the excretory cells to deliver the diodrast into the tubular lumina. Both depress the tubular excretion but by different mechanisms.

Together with Arne Engström an attempt was made to visualize the diodrast microscopically during its passage through the excretory cells. This was accomploshed by means of Engström's micro-X-ray-technique. The results will be described later on.

The substance or substances responsible for the tubular excretion (the "transpositor") were examined chemically. It was prepared from the kidneys of rabbits in which one of the ureters had been ligated and which were given an intravenous diodrast injection one week later. The transpositor was subsequently followed in the ground tissue by its iodine content. It turned out to be an albumin. This work is only recently started and will be reported later on.

Together with Bucht, Werkö and Lagerlöf, the tubular excretion was studied as an energetic process. The oxygen content of the arterial blood of healthy human subjects was compared with that of renal vein blood when a large amount of para-amino-hippuric acid was given. The oxygen consumption of the kidney was very little increased by heavy tubular excretory work. The experiments are described in a paper in this number of this journal.

F. TUDVAD and J. VESTERDAL (Children's Hospital on Martinsvej, Copenhagen): I n u l i n and P A H C l e a r a n c e s i n N e w b o r n I n f a n t s.

Summary:

Technique: Inulin and PAH were injected subcutaneously and determined in samples of urine and capillary blood by a micro-method. 0.2 ml heparin plasma was sufficient for a double assay of both inulin and PAH. After removal of the proteins by a Zomogy precipitation, inulin was estimated by a modification of Kruhøffer's method (a resorcine method), and PAH by Bratton & Marshall's method for the estimation of sulphonamides (modified by C. C. Jensen).

During the first 3 weeks of life the dosage was 0.3 g PAH and 1 g inulin. To infants aged 3 weeks to 2 months twice these amounts were given.

In most of the experiments permanent catheterization was used. This was easily done in girls, but was rather unsuccessful in boys owing to difficulties in emptying the bladder and because pain from the catheterization influenced the kidney function.

55 experiments were performed in infants 1—118 days old with birth weights between 1100 and 3750 g.

Results: Self-depression limit for PAH was between 5 and 10 mg per cent PAH in plasma. Using clearance periods with plasma levels of 1—5 mg per cent we found that during the first days of life the PAH clearance was very low (50—60 ml/min. per 1.73 m² body surface). It increased rapidly during the first weeks although with great individual variations. In the first 3 months it only reached half of the adult value. There was no significant difference between the values for full-term and premature infants. Values for infants with birth weight below 1500 g were near the lower limit of the normal range.

The inulin clearance during the first days of life was 12—14 ml/min. per 1.73 m² body surface. It increased very rapidly during the first weeks, but with great individual variations. The adult value was not reached in the first 4 months. It depended on the birth weight in the same way as the PAH clearance.

The filtration fraction was 0.22—0.55 which was considerably higher than the adult value, indicating hat the tubular function was more immature than the glomerular function. One might expect the contrary, as the glomerular tuft at birth is covered by a high columnar epithelium which gradually flattens.

In a Mongolian idiot the filtration fraction was very high (0.60—0.84), probably indicating a very high degree of immaturity of the kidney. Two other Mongolian idiots did not differ from the normal infants in this respect.

Pain (e. g. by hypodermoclysis of glucose solution) caused a considerable depression of both the inulin and PAH clearances with an almost constant filtration fraction.

In some of the experiments it was possible to increase the inulin and PAH clearances by augmenting the diuresis by intravenous infusion of fluid.

During this increase, which was 50—100 per cent, the filtration fraction remained almost constant suggesting that a number of previously inactive nephrons had been put into action (by opening of the vasa afferentia).

(To be published in Acta pædiatrica.)

I. SPERBER (Institute of Animal Physiology. Uppsala, Sweden): T h e E x c r e t i o n o f s o m e O r g a n i c B a s e s a n d s o m e P h e n o l s a n d P h e n o l D e r i v a t i v e s.

The excretion of some organic bases and of some phenols and their detoxication products has been studied in the chicken. The excretion of phenol, phenol sulphuric ester and phenol glucuronide has also been examined in the goat.

In the chicken a method has been used, which is based on the existence of a renal portal circulation in birds (1). The venous blood returning from the leg passes through the intertubular capillaries in the kidney. The substance to be tested is injected into one leg. If the substance is excreted by the tubules, part of the dose is excreted during its passage from the leg through the kidney, The rest of the dose is equally distributed between the two kidneys. The kidney on the side of injection will then excrete more of the substance when the substance is excreted by the tubules, and the difference between the kidneys is proportional to the efficiency of the tubular excretion. By a special arrangement the urine from each kidney is collected separately.

By the use of this method it has been shown that methylnicotinamide, piperidine, guanidine, and methylguanidine are excreted by the tubules (2). This secretion of organic bases seems to be separate from the secretion of anions, as injection of large doses of hippuric acid or diodrast has no influence.

Glucuronides of some phenols and the sulphuric esters of several phenols are also excreted by the tubules in the chicken (3). This excretion is depressed by hippuric acid. The free phenols are not excreted by the tubules.

The excretion of free phenol and conjugated phenol has been investigated in the goat by the use of the clearance method, using creatinine as a measure of glomerular filtration. The free phenol has a clearance on an average about 20 per cent of the creatinine clearance. Phenol sulphuric ester and phenol glucuronide have far higher clearances, the former substance slightly less, the latter

slightly more than three times the simultaneous clearance of creatinine. It is thus clear that the excretion of these compounds is qualitatively similar in the chicken and the goat.

The investigations have been published in:

1) Zoologiska bidrag från Uppsala, *27, 429.*
2) Ann. Roy. Agric. Coll. Sweden, *16, 49.*
3) Ann. Roy. Agric. Coll. Sweden, *15, 317.*

P. EFFERSØE (Institute of General Pathology, Copenhagen): Comparative determinations of the Creatinine and Thiosulphate Clearances in Kittens.

In man the thiosulphate clearance is identical with the inulin clearance; in dogs and rabbits it is equal to the creatinine clearance, which for these animals is reckoned as a measure of the filtration.

On the other hand, in cats, Jens Bing and the author have found that with low plasma concentrations the thiosulphate clearance is more than twice as high as the creatinine clearance, but that with rising plasma concentrations of thiosulphate this difference approaches zero. This is best explained by assuming that cat tubules are capable of secreting thiosulphate (Acta phys. scand. 15, 231, 1948).

No explanation is forthcoming for this difference between cats and all the other animals tested; but as one of the functions of the kidneys is to excrete the products of metabolism it might be that it is due to dietary differences.

The thiosulphate and creatinine clearances were determined simultaneously on 4 kittens two weeks old and one that was eight weeks old. The kittens had never had anything but mother's milk, but it is a question whether it would not have been better if they had been given milk from one of the animal species whose tubules do not secrete thiosulphate.

These determinations showed that with varying plasma-thiosulphate-concentrations, the ratio of creatinine to thiosulphate clearance varied in exactly the same manner as in adult cats. Accordingly if cat tubules secrete thiosulphate the property is already developed while they are living on milk and before they receive the diet normal for adult cats.

E. BOJESEN (Institute of Medical Physiology, University of Copenhagen): Secretion of Diodrast by the Small Intestine.

I wish to report some preliminary experiments on the power of the small intestine to secrete Diodrast. The reason for the experiments is the analogy wich exists between the physiology of the small intestine and the renal tubule.

Cats were used for the experiments, but a few experiments on dogs have given similar results. Dodrast was analysed by a method published previously (1). According to this no Diodrast iodine is lost by the precipitation of the proteins. At first it was shown that the small intestine allows the passage of Diodrast from the blood to the lumen. An accumulation of Diodrast was observed in diodrast-free plasma injected in the intestinal lumen when Diodrast was injected intravenously. To prove that Diodrast is actively secreted, I have carried out the following experiments to demonstrate that the substance is transported through the cells against a concentration gradient. A narcotized cat (Nembutal) is injected intravenously with Diodrast. After about one hour, a large blood sample is drawn from an artery, the sample is centrifuged, the heparinized plasma is injected with a fine cannula into the lumen of an isolated, rinsed loop of the small intestine. After 15—30 minutes the plasma is squeezed out through a cannula in the end of the loop, and the loop is rinsed with saline and air. In experiments of this kind one always finds an increase in both the concentration and the amount of Diodrast iodine in the lumen of the loop, up to twice the amount injected into the intestine. Experiments made simultaneously on a loop of the jejunum and of the ileum have shown that the same quantities of Diodrast are secreted from the blood to the lumen per unit weight of the two parts of the small intestine. According to these experiments the power of secretion varied much from animal to animal, but it appears in general to be of the order of 15 micrograms Diodrast iodine/kg body weight/min. uneffected by the plasma concentration of Diodrast.

Further investigations on the extent of this intestinal secretion of Diodrast under more varied conditions and on its relation to the secretion of "succus entericus" are planned. They will be published in "Acta physiologica scand.".

Reference:

Bojesen E.: Determination of Diodrast (Diodone) in Blood and Urine according to a Modification of White and Rolfs Method. Acta physiol. Scandinav. 16, 1, 1948.

K. LUNDBÆK and V. POSBORG PETERSEN (Kommunehospitalet, Aarhus): Filtration and Glucose Tm in late Diabetes Mellitus.

Filtration rate (Thiosulphate clearance) and Glucose Tm has been determined in 17 patients with diabetes mellitus of more than 15 years' duration. In 5 patients with proteinuria and hypertension the filtration rate and glucose Tm were considerably reduced, but to about the same degree. The rest of the patients showed a normal or only slightly reduced filtration rate; the glucose Tm, however, in many cases showed a more pronounced depression.

The results reported form part of an investigation now in progress on the clinical and functional pattern in late diabetes mellitus.

S. L. SVEINSSON (Central Laboratory, Ulleval Hospital, Oslo): Renal Excretion of Glycerol. (Acta Physiol. Scandinav. 15, 322, 1948.)

Earlier investigations on the excretion of glycerol in rabbit kidneys (Holst 1943) have shown that the excretion per cent of glycerol (the amount of glycerol excreted in per cent of the amount filtered) was constant and independent of the glycerol concentration in the blood and of the degree of diuresis. These observations have been controlled in humans and the results presented in the present paper. The experiments have confirmed previous findings as long as the glycerol concentration in the blood is above 20—30 mg per cent. When the glycerol concentration is lower, however, it was found that the excretion per cent decreased with decreasing concentration of glycerol in the blood. There is strong evidence for the assumption that the threshold value of blood glycerol is about 8—10 mg per cent. These findings are interpreted as indicating that the reabsorption of glycerol in the kidneys is partly an active absorption, partly diffusion, but since the Tm of glycerol is very low, the active reabsorption will have an observable effect on the excretion per cent only when the glycerol concentration in the blood is low. The excretion per cent thus decreases when the blood glycerol decreases, indicating that a constantly higher per cent of the filtered glycerol is returned to the blood from the tubuli.

The observation that glycerol excretion is independent of diuresis may be explained by the assumption that the diffusion of glycerol from the tubuli to the blood takes place only in the proximal part of the tubulus while the distal part, where the facultative water absorption takes place, must be assumed to be impermeable to glycerol.

References:

Holst, E.: Glycerinundersøgelser til belysning af teorien om resorptionsfosforyleringen. Diss. København, 1943.

M. BJØRNEBOE, S. DALGAARD-MIKKELSEN and F. RAASCHOU (Medical Department III, Kommunehospitalet, Copenhagen): Does the Filtration(Thiosulphate Clearance) Differ at Different pH Values in the Urine?

In connection with the discussion about factors influencing the glomerular filtration, some observations of the value of the thiosulphate clearance at different pH values of the urine may be briefly mentioned.

In a series of examinations of the excretion of salicylic acid at different pH values in the urine, we had at the same time the opportunity to follow the thiosulphate clearance (Table I). Three duplicate experiments were made in 3 normal subjects in such a manner that the examinations were first made at urine pH about 4.8 to 6.4 and then — a week later — at pH from 7.6 to 8.2. In order to produce an acid reaction of the urine, the subjects were treated in advance for about 3 days with ammonium chloride, and for 3 days before the experiments with alkaline urine they were given sodium bicarbonate by mouth. In the course of the actual experiments sodium salicylate was administered intravenously in slowly increasing doses with a view to the main object of the experiments; sodium salicylate was administered in uniform dosage in the acid and the alkaline experiments. As a rule, one experiment comprised from 6 to 9 clearance periods.

The table shows the average thiosulphate clearance values in the three duplicate experiments; further the number of clearance periods on which these average figures are based, and the pH ranges in the urine within which the thiosulphate clearance was determined. It appears that *the thiosulphate clearance on the average is higher in the experiments with alkaline urine.* As the differences appear to be moderate, we have asked a statistician to undertake a computation with due regard to the many clearance periods which form the basis of the results, and to the fact that the three duplicate experiments tend in the same direction. Considered as a whole, the total difference between the three duplicate experiments is more than four times the corresponding mean error and, consequently, the question of a mere coincidence need not be considered.

Table I.

Thiosulfate clearance (ml/min) Average	Number of clearance periods	pH.	Thiosulfate clearance (ml/min) Average	Number of clearance periods	pH.
119.5	9	4.9 — 5.5	145	9	7.6 — 7.8
135	6	4.8 — 5.8	145	6	7.9 — 8.1
93	8	5.5 — 6.4	103.5	6	8.1 — 8.2

It would be too early to attempt any explanation of these findings as long as we do not know how the blood pressure, plasma proteins, colloid osmotic pressure, the pH of the plasma, and the patient's weight are influenced under the conditions of the two experiments.

It may be pointed out that the alkaline experiments were preceded by a higher sodium load to the organism than the experiments with acid urine, and that it has been shown by Shannon in experiments with dogs that the glomerular filtration rises in the course of increasing sodium loads.

Thiosulfate was determined by the method of Brun (J. of Lab. and Clin. Med. 1949, in press); the urine pH has no influence on the analytical result of a known solution of thiosulfate.

C. BRUN, T. HILDEN and F. RAASCHOU (Medical Department III, Kommunehospitalet, Copenhagen): Examinations of the Delay-time of the Kidney.

By *delay-time* is understood the time passing from the formation of the glomerular filtrate till the corresponding portion of urine reaches the bladder — or is evacuated through a catheter.

In order to illustrate the duration of the delay-time we have performed injection experiments with sodium thiosulphate, and with a special stepped-off injection technique we managed to maintain a fairly constant thiosulphate concentration in the arterial blood from the time when the injection was given until about 15 minutes later. The urine was collected every minute through a catheter (V) and its concentration of thiosulphate (U) was determined. The curve representing the total excretion of the substance (U × V) showed the following course (Fig. 1): First, about 3 minutes pass before thiosulphate can be demonstrated in the urine ("*appearance-time*"); then follows a period of about 4 minutes during which the total excretion of thiosulphate rises rapidly; about 7 minutes after the injection was given the curve showing the total excretion displays a bend and then follows a horizontal, plateau-shaped course.

The explanation of the course of the curve is supposed to be as follows: (1) The appearance-time indicates the time passing from the injection of the substance till the first quantities of thiosulphate reach the bladder. (2) The rapid rise of the total excretion may be imagined to be due to: (a) first, it must indicate that urine containing thiosulphate is mixed with thiosulphatefree urine in the collecting tubules, the renal pelvis and the ureter, so that the bend of the curve marks the time when the mixing has been completed. (b) Secondly, there is the possibility that the time passing from the filtration process till the urine appears in the collecting tubules varies in the different nephrons, which is supported by the fact that anatomically, the various nephrons are known to be of different lengths.
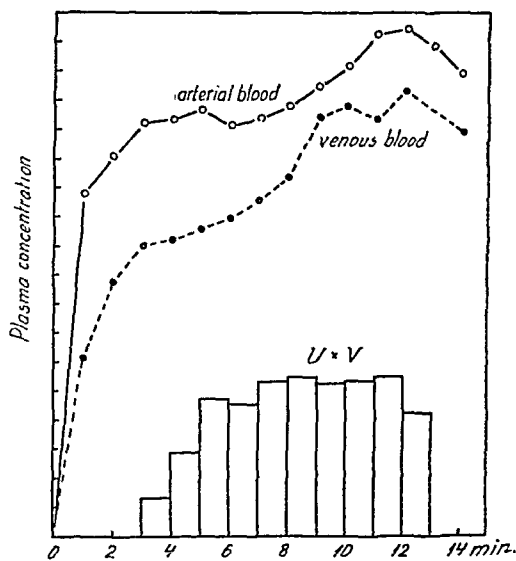
Fig. 1.

Thus, the urine collected during a period of one minute consists of urine portions produced in the kidneys over a certain, longer period, which, in the above mentioned experiments, is about 4 minutes.

If one needs a correction for the delay-time as in experiments with rapidly changing, plasma concentrations we are inclined to consider that in our curve this time is represented by the period passing from the moment, when the substance reaches the kidneys by the blood till the middle of the rapidly rising curve of the total excretion.

Owing to the different rate of the flow through the dead space of the kidneys, the delay-time is to some extent dependent on the output of urine, as it is longer at a low output and shorter at a high one. Examinations of this dependence are going on. At the present moment it can be stated that at urine volumes around 2—5 cc/min. the delay-time is about 5 minutes.

T. HILDEN (Blegdamshospitalet Copenhagen, Denmark): On the Renal Mechanism for Excretion of Water and Salt.

A brief survey is given of the few facts known about renal excretion of salt and water. The hypothesis on which present investigations of these problems are based is also discussed.

Variations in diuresis between 0.4 cc/per min. and about 20 cc/per min. is, in man, supposed to be due to variations in the reabsorption of water in the distal tubulus, which in turn is caused by the variable effect of the posterior pituitary hormone, A. D. H.

Diuresis' below 0.4 cc/per min. is due to a decrease in the filtration. Diuresis over about 20 cc/per min. is induced by administration of osmotic active substances, which are excreted in the urine, as all these substances are thought to restrain the absorption of water in the proximal tubulus.

As in the case of water, it is believed that a considerable, but isotonic, salt reabsorption takes place, in the proximal tubulus while the salt reabsorption in the distal tubulus is able to take place in spite of great differences in the concentration such as by the production of a hypotonic urine.

Variations in the excretion of NaCl can take place either by changes in the filtered amount or by changes in the reabsorption. Some investigations imply that variations in the administered amount of NaCl provoke parallel changes in the filtered amount of NaCl. It is not known whether variations in the amount of reabsorbed NaCl also occur, for instance influenced by varying production of the adrenal hormone.

In normal men about 1100 g of NaCl is filtered daily; of which 1090—1095 g is reabsorbed, as the excretion as well known, is generally about 5—10 g. From this it is obvious that even small variations in the filtered or reabsorbed amount may provoke considerable changes in the excretion of NaCl. In pathological cases an unequal influence of the glomerular and the tubular function might influence the excretion of NaCl through the kidneys.

H. EDER (The Rockefeller Institute, New York):
The Mechanism of Nephrotic Edema.

E. MØLLER-CHRISTENSEN (Copenhagen):
Diuresis of Rats after Peroral Ingestion of Water in Massive Doses.

Five experiments were made with rats (weight about 175 g) which were gradually trained to tolerate water given by mouth in continuously increasing quantities. By administering a standard quantity of water (50 ml in 5 ml portions at half hour intervals) before and after habituation it is possible to observe 1) a marked increase in the diuresis and 2) a much more rapid onset of urine excretion.

Two other experiments showed that repeated ingestion of large quantities of water causes a reduced excretion of NaCl through the urine.

These results agree with those of earlier investigators (GAUNT et alii) and like them, the author considers that the adrenal cortex plays no small role in the habituation of the animals to large quantities of water.

O. J. MALM (Central Laboratory, Ulleval Hospital, Oslo): Studies on the Recovery of Renal Function after Resection of the Single Kidney in Renal Tuberculosis.

The technique for partial resection of the kidney, developed by chief surgeon Carl Semb of Surgical Department III, Ulleval sykehus, Oslo (Acta Chir. Scandinav. 98, 475, 1949) has made it possible to perform resection of the kidney in patients where the other kidney has been removed, or is without function due pyonephrotic destruction with complete stenosis of the ureter.

During the last year 3 patients with localised tuberculous destruction in the remaining kidney have been resected by Dr. Semb. These cases have provided an unusual opportunity of following the recovery of kidney function during the immediate postoperative period, and studies of inulin, endogenous creatinine, urea and p-aminohippuric acid clearances have been made 1—5 months after the operation. T_{MAX}PAH has also been studied in 2 cases. In one case the kidney vessels were clamped for 50 minutes during the resection of the lower pole of the kidney. In 2 cases manual compression of the kidney substance supplemented by tourniquet compression of the tissue close to the line of resection was relied upon to control the bleeding from the kidney parenchyma. From 25—30 per cent of the kidney substance was removed, and in addition an unknown quantity of the parenchyma was rendered functionless by the closure of the wound in the kidney. The clamped kidney showed the most pronounced depression of its function in the postoperative period, B. U. N. reached 177 mg per cent on the 8th day after operation. 50 per cent preoperative renal function was attained on the 26th day.

The 2 nonclamped kidneys recovered more quickly. B. U. N. did not exceed 75 mg per cent after the operation and 50 per cent of preoperative function was attained in less than 3 weeks.

The further course during the 4—5 months after the resection has been characterized by a slight rise in the glomerular filtration from about 30—35 ml/min. T_{MAX}PAH has risen in the 2 patients examined. Calculated as per cent of normal average, T_{MAX}PAH 5 months after operation exceeds 60 per cent compared to a glomerular filtration of 37 per cent and a urea clearance of 47 per cent of normal average.

The ratio urea clearance/inulin clearance has risen from 0.6, 24 days after resection to 0.8 four months after operation in the clamped and in one of the nonclamped kidneys. The other nonclamped kidney showed a ratio of 0.87 on the 17th day, 0.97 3½ months after operation, and 0.74 five months after operation. A slight proteinuria persists in all cases, hyaline and granular casts in small amounts have been found regularly. There is no polyuria after the second month after opera-

tion, B. U.N. (fasting) is about 20—25 mg per cent. The patient who had his renal vessels clamped, developed a moderate hypertension (blood pressure 150—170/105) which became evident 6—8 weeks after operation and still persists 5 months later. The 2 nonclamped patients have normal and unchanged blood pressure. The cases will be reported in detail in this journal.

O. POVLSEN (Bispebjerg Hospital, Copenhagen): Bladder-Neck Obstruction; Back Pressure; Kidney Function.

In 1941, the author found (Om Cystometri hos prostatikere. Thesis, University of Copenhagen, 1941) that 80 per cent of the patients with hypertrophy of the prostate have hypertonic bladder, and that transurethral resection of the prostate does not lead to any changes of the hypertony; this is shown by making another cystometry shortly after the operation.

In 1944, J. C. Christoffersen found (Transuretral Resection, Thesis, Univers. of Copenhagen) that the hypertony persists several years after the operation, probably for ever.

Ectomy, however, reduces hypertony.

The question is: Does hypertony of the bladder cause damage to kidney function? Several authors think so.

A new group of 203 patients with benign obstruction of the bladder-neck has been investigated 1) with regard to bladder function (compensation, decompensation) 2): the shape and function of the pelvis by excretory urography, and 3) kidney function tests.

These investigations have shown that damage to kidney function in patients with hypertrophy of the prostate generally occurs in cases with decompensation of the bladder, whereas the functions remain undisturbed in cases with compensation of the bladder.

Hypertony of the bladder wall is the best protection against back-pressure damaging the kidney of patients with bladder-neck obstructions.

M. SIMONSEN (Institute of General Pathology, Copenhagen): Kidney Transplantations.

Total removal of a kidney and reimplantation in the same animal with vascular and ureter anastomoses, a so-called autotransplantation, has been successfully performed by several workers, especially by Carrel. Several instances have been published where such a kidney functions normally for years, but the great majority of autotransplantations perish long before. When a kidney has been transplanted from another animal of the same species — homotransplantation — the result has usually been cessation of the renal function after a few days, and in rare cases after a few weeks. The literature contains no report of any attempt, serologically or biochemically, to clarify the cause of the perishing of the homotransplant in cases where it could not be explained by vascular thrombosis or by operative complications. The pathoanatomical descriptions are sparse and mutually highly contradictory.

In my own experiments I have made six autotransplantations and two homotransplantations on dogs, transplanting the kidney into the renal position after excision and weighing. For vascular anastomoses I employed vitallium or polyethylene tubus of Blakemore-Lord's model. Polyethylene tubes of my own design were used for ureter anastomosis. In most cases the contralateral kidney was removed in the same operation as the transplantation.

The renal function was resumed after the operation in every case, but all the animals died subsequently. The best of the autotransplants perished after three weeks from thrombosis in the renal artery, whereafter malignant hypertension developed. The two homotransplants perished after 5 and 7 days respectively. In these latter cases, post mortem examination revealed neither vascular thrombosis nor other postoperative complications, but on removal the kidneys weighed about twice as much as on transplantation. On the other hand, all the autotransplanted kidneys retained their weight unchanged or had perhaps lost a couple of grammes, except in one case where pyonephrosis developed. Histologically, too, the two homotransplants were found to be significantly different from the autotransplants, all cells exhibiting widespread toxic degeneration, especially of tubules, intra and intercellular oedema and secondary inflammatory infiltration, chiefly of lymphocytes. The changes were not of a tissue-allergic character. The autotransplants were either histologically normal (at death from accidental complications 5—16 days after transplantation) or showed localized changes caused by ischaemia. Biopsy of the spleen during the operation and after death showed no plasma cell proliferation as a sign of antibody formation. Nor did the complement fixation test show any sign of antibody formation against the transplant. The blood of donors and receptors showed no type difference in agglutination tests. In supplementary immunological experiments 7 rabbits were injected with dog-kidney suspension and one dog with rabbit-kidney suspension. This caused the formation of antibodies which gave in either case complement fixation both with dog-kidney and rabbit-kidney suspensions. In no case, however, was there any increase of the blood urea or blood pressure or any urine change, and the kidneys of the immunized animals were histologically normal.

Conclusion: Nothing has yet been found to show that the biological incompatibility in the homotransplantation of kidneys is the result of differences in antigens.

To be published in Acta path. Scandinav.

LEVIN NIELSEN, K. TRAUTNER and J. MOUSTGÅRD (Kommunehospitalet and State Research Laboratory, Department of Animal Physiology, Copenhagen): Kidney Transplantations.

Four autotransplantations have been made on dogs; of these one was succesful, and the dog is alive and well ten months after the operation. It has, however, been possible to conclude, by determining the clearance values, that the kidney no longer reacts normally, i. e. by showing a rise in the clearance when the dog is fed on meat (65 g per kg). Blood pressure, blood urea and weight are normal.

Eight homotransplantations have been made on dogs and 3 on goats. All these animals died, after surviving the transplantation from 2 to 14 days. In 10 of 11 cases the opposite kidney was removed.

The length of time the animals survived increased steadily. The cause of death, apart from the first cases, was thrombosis of the renal artery. In 2 cases however, the cause of death was hydronephrosis.

P. HEDLUND (State Bacteriological Laboratory and IVth Medical Service, S:t Eriks Hospital, Stockholm): The Pathogenesis of Glomerulonephritis.

An attempt was made to correlate the appearance of experimental glomerulonephritis in rabbits and mice with the presence of autoantibodies against kidney tissue of the same species.

After reviewing various methods for inducing kidney lesions (1, 2) it was pointed out that Masugi-nephritis, which is a form of passive immunization, is the only experimental nephritis which is histologically and clinically comparable to human glomerulonephritis (Smadel (3)). Previous attempts, making use of active immunization, have not been successful in inducing acute glomerulonephritis. On the other hand it has been possible to demonstrate antibodies against kidney tissue of the same species after injection of such tissue plus products of streptococci or staphylococci (Schwentker & Comploier (4), Cavelti (5)).

In the experiments referred to here, animals were injected subcutaneously with a mixture of kidney tissue of the same species and living hemolytic streptococci. Such an infectious focus was established only once in each animal.

Fig. 1 shows the serological reactions in such an experimental animal. The antistreptolysin titer rises and remains at a high level for a prolonged period. The animal reacts with the formation of acute phase protein. The serum agglutinates a strain of hemolytic streptococci belonging to the same serological group A as the injected organisms. Collodion particles, sensitized with rabbit kidney tissue, are agglutinated as evidence that antibodies against kidney tissue have been formed. It is of interest that the acute phase protein, the agglutination against haemolytic streptococci, and the collodion titer are not correlated in time.

Table I.

Focus		Nr.	Anti-Streptolysin-titer	Acute phase Protein titer	Agglutination titer	Collodion-titer
I	Kidney emulsion Hemol. sreptoc. Agar	1	560	+8	1/40	1/80
		2	<25	+16	1/20	>1/640
		4	200	+8	1/80	1/40
		5	180	+4	1/80	1/160
II	Hemol. streptoc. agar. . . . . . . kidney emulsion agar	6	<25	+2	0	0
		7	50	+4	1/320	0
		8	800	+4	1/40	0
III	Hemol. streptoc. agar	9	1100	+4	1/160	(1/20)
		10	800	+8	1/320	0
IV	Kidney emulsion agar	11	<25	—	0	1/20
		12	25	—	0	0
		13	<25	—	0	0
V	Hemol.streptoc(16008) kidney emulsion agar	14	1100	—	1/40	1/160
		15	1100	+8	1/1600	>1/640

16008 = a strain of hemolytic streptococci. group A.

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the view that a chronic focal infection may be responsible for the positive streptococcus agglutination in chronic nephritis and rheumatoid arthritis. — In recent years the possibility of unspecific increased agglutination capacity in these sera has been discussed.

A more detailed report of the investigation will be given in a later number of this journal.

J. RUDEBECK (County Hospital, Örnsköldsvik, Sweden): Prognosis of acute glomerulonephritis.

Only acute postinfections glomerulonephritis is discussed.

In about 80 per cent complete recovery occurs. In 5 per cent or somewhat more death follows in the acute or subacute stage of the disease. In 10 to 15 per cent a chronic progressive glomerulonephritis develops. The chance of complete recovery may roughly be given as 90 per cent for patients under 30 years, but hardly 50 per cent for patients over 50 years.

In cases where all symptoms of nephritis have disappeared relapses may occur, but the risk of a future chronic nephritis is extremely small. Slight urinary symptoms persisting for 6 months do not appear to imply any particular reduction in the chanse of recovery, but if they persist for a year or more, they may imply a greater risk of chronic nephritis. More serious urinary symptoms with or without hypertension aggravate the prognosis even after a duration of a couple of months.

In a material of 114 cases, carefully examined and followed up, an investigation was made of he clinical course during the first twelve months ifter the onset of the disease. 88 cases recovered

and 26 developed chronic nephritis. The main results were as follows.

In cases which recovered, proteinuria of more than 5 per mille Esbach was rarely observed in the initial stage of the disease; after the first month, proteinuria hardly ever exceeded 1 per mille. In cases which became chronic, marked proteinuria was frequently seen throughout the first year of the disease.

In the cases which recovered, the sedimentation rate after one month rarely exceeded 50 mm per hour: after six months the value exceeded 30 mm only in exceptional cases. In cases which became chronic, a high sedimentation rate was frequently observed throughout the first year of the disease.

In the cases which recovered, a reduced kidney function (as measured by the urea clearance test and the concentration test) was rarely seen after the sixth month of the disease, though frequently in cases which developed into chronic nephritis.

In cases of glomerulonephritis, careful and repeated clinical examinations from the very onset of the illness would probably be of great help to a relatively early prognostic evaluation, which is of particular importance in protracted cases.

(For more extensive publication, including diagrams, see Nord. med. 41, 680, 1949.)

N. ALWALL (Medical Dept., University Hospital, Lund, Sweden): Treatment of Uremia with Artificial Kidney.

See:
1. On the artificial Kidney I—XIII. Acta med. Scandinav. 1947—1949.
2. Verhandl. Kongr. Deutsch. Urolog. Gesellschaft. München 29, IX, 1949.

INDEX OF AUTHORS

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